

**METHODS FOR THE PREVENTION AND TREATMENT OF PARASITIC
INFECTIONS AND RELATED DISEASES USING CPG OLIGONUCLEOTIDES**

Related Applications

This application claims priority under Title 35 §119(e) of the United States Provisional
5 Application No. 60/084,512, filed May 6, 1998, and entitled "IL-12 and IFN- γ Dependent
Protection of Mice Against Malaria Conferred by CpG DNA", the entire contents of which are
incorporated herein by reference.

Field of the Invention

The present invention relates to the use of immunostimulatory CpG oligonucleotides
10 in the prevention and treatment of parasitic infection and disease.

Background of the Invention

Parasites are organisms which depend upon other organisms in order to survive and
thus must enter, or infect, another organism to continue their life cycle. The infected
organism, i.e., the host, provides both nutrition and habitat to the parasite. Although in its
15 broadest sense the term parasite can include all infectious agents (i.e., bacteria, viruses, fungi,
protozoa and helminths), generally speaking, the term is used to refer solely to protozoa,
helminths, and ectoparasitic arthropods (e.g., ticks, mites, etc.). Protozoa are single celled
organisms which can replicate both intracellularly and extracellularly, particularly in the
blood, intestinal tract or the extracellular matrix of tissues. Helminths are multicellular
20 organisms which almost always are extracellular (the exception being *Trichinella* spp.).
Helminths normally require exit from a primary host and transmission into a secondary host in
order to replicate. In contrast to these aforementioned classes, ectoparasitic arthropods form a
parasitic relationship with the external surface of the host body.

Rarely is the parasite-host relationship symbiotic, with both the parasite and the host
25 benefiting from the interaction. Instead, parasitic infections, particularly helminthic
infections, and the diseases to which they give rise, are chronic conditions, due to the initial
asymptomatic presence of some parasites. In extreme instances the infection, and the related
disease, are acute and, if left untreated, can be lethal. These latter instances represent a small
proportion of total parasitic infections, most probably because the parasite is ultimately
30 dependent upon a viable host in order to propagate.

Parasites are capable of infecting almost any tissue or cell type, however, depending
on the particular parasite, they tend to preferentially target a subset of cells including, in

humans, red cells, fibroblasts, muscle cells, macrophages and hepatocytes. For example, the protozoan *Entamoeba histolytica* which is found in the intestinal tract and propagated by contact with host feces, can migrate across the intestinal mucosal lining to infect other bodily tissues such as the liver eventually forming amoebic abscesses. Other parasites can be
5 transmitted via intermediate hosts such as mosquitoes. Ectoparasitic arthropods are a nuisance for household pets (e.g., dogs, cats) and, more importantly, can contribute to wasting syndromes and act as a vehicle for the transmission of other infections (such as babesiosis and theileriasis) in agricultural livestock.

Malaria is the most prevalent parasitic disease in humans. It is estimated that malaria-
10 causing parasites such as *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium knowlesi* and *Plasmodium ovale* result in an estimated 300-500 million new infections and 1.5 to 2.7 million deaths annually in less developed areas of the world (WHO, 1995). In addition, tens of millions of travelers from countries, where malaria is not endemic, visit countries where it is, and many of these travelers succumb to illness during their travels
15 or after returning home. In the latter case, there is a particular risk of failure to rapidly diagnose and initiate treatment, owing to the lack of experience with the disease by local physicians.

Other parasitic infections in humans include schistosomiasis, filariasis, hookworm, ascariasis, leishmaniasis, trichinosis, Chagas' disease and African trypanosomiasis.

20 In addition to the human health risks, parasites also pose a considerable risk to agricultural livestock and domestic and wild animals. Agricultural livestock and in some cases zoo animals are ripe targets for widespread transmission of parasitic diseases for two major reasons. First, livestock usually live in such close quarters thereby facilitating the transmission of a parasite to an entire flock or herd. Second, because many enteric parasites
25 eventually exit the body in feces which invariably litter a grazing field for animals, the likelihood of transmission and widespread infection is high. Thus the maintenance of a parasite free environment through prevention of parasitic infections would be highly desirable in these circumstances.

The elimination of parasites by the immune system is usually incomplete due in part to
30 the complex and varied life cycles of parasites which consist of antigenically distinct developmental stages. The immune response to parasitic invasion is generally not humoral (i.e., antibody based) and thus immunological memory does not usually follow from an

infection. As a result, infected individuals do not develop an immunity to the parasite and continue to be susceptible to future infections.

The treatment and prevention of parasitic infection has traditionally depended on the discovery of drugs targeted against the particular parasite or a carrier of the parasite, such as mosquitoes (e.g., insecticides). Although historically productive, many of the parasites, particularly those that cause malaria, have now developed resistance to such drugs and there are few new drug candidates on the horizon (Hoffman and Miller, 1996, Marsh, 1992). Thus new and more effective methods to prevent and treat this widespread and serious disease are required. Considerable effort has been put into the development of vaccines designed to induce specific anti-parasite immune responses. While there has been substantial progress in this endeavor, no anti-malarial vaccine has ever been licensed.

Summary of the Invention

The invention relates to the use of CpG oligonucleotides in the prevention and treatment of parasitic infections and related diseases.

In one aspect, the invention relates to a method for preventing a parasitic infection in a subject comprising administering to the subject at risk of being infected with a parasite an effective amount, for preventing a parasitic infection, of an oligonucleotide having a sequence including at least the following formula:



wherein the oligonucleotide includes at least 6 nucleotides wherein C and G are unmethylated and wherein X_1 and X_2 are nucleotides prior to exposure to a parasite.

In one embodiment, the oligonucleotide is 6 to 100 nucleotides in length. In another embodiment of the invention, X_1 in the above formula is selected from the group of A, T or G. In yet another embodiment, X_2 in the above formula is selected from the group consisting of A, C or T.

In some embodiments of the invention, the subject at risk of being infected with a parasite is a human. In still other embodiments the subject is non-human. In still further embodiments, the invention is directed towards a subject selected from the group consisting of a cat, dog, cow, pig, sheep, horse, chicken, duck, goose, fish, goat, mouse, rat, gerbil, rabbit and a zoo animal.

In one embodiment of the invention, the subject is at risk of infection with an

intracellular parasite. In another embodiment, the parasite is an obligate intracellular parasite. In still a further embodiment, the method of the invention is directed towards the prevention of infection by the following parasites: *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium knowlesi*, *Babesia microti*, *Babesia divergens*, *Trypanosoma cruzi*, *Toxoplasma gondii*, *Trichinella spiralis*, *Leishmania major*, *Leishmania donovani*, *Leishmania braziliensis* and *Leishmania tropica*. In another embodiment, the method is directed towards the prevention of infection by the following parasites: *Trypanosoma gambiense*, *Trypanosoma rhodesiense* and *Schistosoma mansoni*. In preferred embodiments, the method is directed towards the prevention of infection with parasites which cause malaria.

In one embodiment of the invention, the subject is also administered an effective amount of one or more non-CpG oligonucleotide therapeutic agents. In preferred embodiments, the non-CpG oligonucleotide therapeutic agent is a parasiticide. In other preferred embodiments, the non-CpG oligonucleotide therapeutic agent is selected from the group consisting of IL-1, IL-6, IL-12, IL-15, IL-18, IFN- γ , TNF- α , GM-CSF, CD40 ligand and Flt3 ligand. In some embodiments in which IL-12 and IFN- γ are administered, IL-12 is administered prior to IFN administration.

In one embodiment of the invention, the oligonucleotide is administered more than once. In other embodiments, the oligonucleotide is administered at least 7 days prior to a parasite infection. In still other embodiments, the oligonucleotide is administered at least 2 days prior to a parasite infection. In still further embodiments, the oligonucleotide is administered at least 24 hours prior to a parasite infection.

According to some embodiments of the invention, the oligonucleotide is administered orally, mucosally, transdermally, subcutaneously, parenterally, or by inhalation. In other embodiments, the oligonucleotide is administered in a sustained release vehicle. In still further embodiments, the sustained release vehicle is a liposome.

In another aspect, the invention relates to a pharmaceutical preparation comprising the oligonucleotide having a sequence including at least the following formula:



wherein the oligonucleotide includes at least 6 nucleotides wherein C and G are unmethylated and wherein X_1 and X_2 are nucleotides, at least one therapeutic agent and a therapeutically

acceptable carrier. In another aspect, the invention relates to a kit comprising the pharmaceutical preparation comprising the oligonucleotide of the invention with the above formula, a non-CpG oligonucleotide therapeutic agent in a therapeutically acceptable carrier in at least one container and instructions for use wherein the oligonucleotide and the
5 therapeutic agent are in separate containers.

In some embodiments, a sustained release device is used to deliver the compounds of the invention. In some embodiments, a sustained release device comprising the oligonucleotide of the invention and a polymer capable of release for at least 7 days is used. In other embodiments, a sustained release device comprising the oligonucleotide of the
10 invention and a polymer capable of release for at least 10 days is used. In still other embodiments, a sustained release device comprising the oligonucleotide of the invention and a polymer capable of release for at least 30 days is used. And in still further embodiments, a sustained release device comprising the oligonucleotide of the invention and a polymer capable of release for at least 60 days is used.

15 In another aspect of the invention, a method is provided for treating a subject infected with a parasite comprising administering to the subject an effective amount for treating a parasite infection of an oligonucleotide having a sequence including at least the following formula:



20 wherein the oligonucleotide includes at least 6 nucleotides wherein C and G are unmethylated and wherein X_1 and X_2 are nucleotides. In some embodiments, the treatment method involves the use of an oligonucleotide which is 6 to 100 nucleotides in length. In still other embodiments the treatment method uses oligonucleotides in which X_1 in the above formula is selected from the group of A, T or G. In yet other embodiments, the treatment
25 method uses oligonucleotides in which X_2 in the above formula is selected from the group consisting of A, C or T.

In some embodiments of the invention, the method of treatment is directed to a human subject. In other embodiments, the method of treatment is directed to a non-human subject. In certain embodiments the subject having a parasitic infection is selected from the group
30 consisting of a cat, dog, cow, pig, sheep, horse, chicken, duck, goose, fish, goat, mouse, rat, gerbil, rabbit and a zoo animal.

In one embodiment, the method of treatment is directed to a subject infected with an intracellular parasite. In another embodiment, the method of treatment is directed to a subject infected with an obligate intracellular parasite. In preferred embodiments, the method of treatment is directed to a subject infected with *Plasmodium falciparum*, *Plasmodium ovale*,
5 *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium knowlesi*, *Babesia microti*, *Babesia divergens*, *Trypanosoma cruzi*, *Toxoplasma gondii* or *Trichinella spiralis*. In other embodiments, the method of treatment is directed to a subject infected with *Trypanosoma gambiense*, *Trypanosoma rhodesiense* and *Schistosoma mansoni*. In even more preferred embodiments, the method of treatment is directed to a subject infected with a parasite which
10 causes malaria.

In certain embodiments, the subject having a parasitic infection is also administered an effective amount of one or more non-CpG oligonucleotide therapeutic agents. In some embodiments, the non-CpG oligonucleotide therapeutic agent is a parasiticide agent. In still other embodiments, the subject having a parasitic infection is also administered an
15 non-CpG oligonucleotide therapeutic agent selected from the group consisting of IL-1, IL-6, IL-12, IL-15, IL-18, IFN- γ , TNF- α , GM-CSF, CD40 ligand and Flt3 ligand. In certain embodiments in which the subject having a parasitic infection is administered IL-12 and IFN- γ , IL-12 is administered prior to IFN administration.

In other embodiments, the oligonucleotide is administered more than once to the
20 subject having a parasitic infection. In still other embodiments, the oligonucleotide is administered within 24 hours of parasite infection to the subject having a parasitic infection. In still further embodiments, the oligonucleotide is administered within 48 of parasite infection. In yet other embodiments, the oligonucleotide is administered within 7 days of parasite infection.

25 In some embodiments, the oligonucleotide is administered orally, mucosally, transdermally, subcutaneously, parenterally, or by inhalation to a subject having a parasite infection. In yet another embodiment, the oligonucleotide is administered in a sustained release vehicle to a subject having a parasitic infection. In a further embodiment, the sustained release vehicle is a liposome.

30 It is to be understood that for all aspects and embodiments of the invention as described herein, the oligonucleotide referred to is one having the sequence including at least

the following formula:



wherein the oligonucleotide includes at least 6 nucleotides wherein C and G are unmethylated and wherein X_1 and X_2 are nucleotides.

5 **Detailed Description of the Preferred Embodiments**

The invention is related to the discovery that administration of unmethylated CpG containing oligonucleotide to a subject prevented parasitic infection in the subject. As used herein, the terms "CpG containing oligonucleotides" and "CpG oligonucleotides" are used interchangeably. The observations on which the invention is premised demonstrate the ability
10 of CpG oligonucleotides to stimulate the innate immune system thereby providing protection against parasites such as those causing malaria and treatment of parasitic infection.

In one aspect, the invention involves a method for preventing parasitic infection in a subject. Parasitic infection arises from exposure to parasites which can occur in a number of ways. Transmission is possible through contact with bodily fluids, tissues or waste products
15 from infected individuals, or through contact with intermediary hosts such as insects (e.g., insect bites). Individuals who are infected with parasites can be identified based on physical symptoms and/or clinical findings including the observation of parasitic bodies or debris in samples of bodily fluids, tissues or waste.

In one aspect, the methods of the invention involve administering to a subject, at risk
20 of being infected with a parasite, a CpG containing oligonucleotide in an amount effective to prevent a parasitic infection in the subject. As defined herein, an individual "at risk of being infected with a parasite" is one who has any risk of exposure to an infectious parasite such as conditions or environments in which parasite infections are common, including contact with an infected individual. A subject is at risk of parasitic infection if there is a possibility that the
25 subject will be exposed or come in contact with another individual either known to be or later diagnosed as suffering from a parasitic infection. For example, an individual anticipating travel to a region in which parasitic infections are endemic is considered a person at risk of being infected with a parasite. The prevalence in some countries of parasites, and the diseases to which they give rise, increases the likelihood that travelers, workers and military personnel
30 assigned to these regions will be at risk of parasitic exposure and subsequently, suffer from a parasitic infection.

In addition to the use of the CpG oligonucleotide for prophylactic treatment, the invention also encompasses the use of the CpG oligonucleotide for the treatment of a subject having a parasite infection. A "subject having a parasite infection" is a subject that has been exposed to an infectious parasite and has acute or chronic detectable levels of the pathogen in the body. The CpG oligonucleotide can be used to mount an innate immune response that is capable of reducing the level of or eradicating the infectious pathogen (i.e., parasite). The innate immune response does not involve an antigen and is thus useful against any type of pathogen. In addition to the innate immune response the CpG oligonucleotide may also enhance an antigen specific immune response if an antigen is administered with the CpG oligonucleotide. An antigen specific immune response, however, is not required for prophylactic or treatment purposes according to the invention. An infectious parasitic disease, as used herein, is a disease arising from the presence of a parasite in the body.

In preferred embodiments, the subject has been exposed to malaria causing *Plasmodium* spp. In other embodiments, the subject has been infected with *Trypanosoma cruzi*, *Trichinella spiralis*, *Babesia* spp. or *Toxoplasma gondii*. When used as a mode of treatment, the CpG oligonucleotides of the invention can be administered following suspected or confirmed parasite exposure. As will be discussed herein, a subject infected with a parasite often times exhibits a set of symptoms which can be used to identify the presence of the parasitic infection and in some instances, the particular parasite involved.

Parasitic infections which the compounds and methods of the invention seek to prevent and treat include those occurring in humans and non-human vertebrates. According to some embodiments, the methods of the invention are directed towards human subjects. In yet other embodiments, the methods of the invention are directed towards non-human vertebrates including agricultural livestock and domesticated and wild animals, such as, for example, cattle, horses, swine, goats and sheep, poultry and other winged vertebrates; rabbits, dogs, cats, ferrets and fish. Non-human vertebrates which exist in close quarters and which are allowed to intermingle as in the case of zoo and research animals are also embraced as subjects for the methods of the invention. Zoo animals such as the felid species including for example lions, tigers, leopards, cheetahs, and cougars; elephants, giraffes, bears, deer, wolves, yaks, non-human primates, seals, dolphins and whales; and research animals such as mice, rats, hamsters and gerbils are all potential subjects for the methods of the invention.

The methods of the invention when used prophylactically embrace the prevention of infection from parasitic species to which the vertebrate subjects are vulnerable. Most parasites are host-specific or have a limited host range, i.e., they are able to infect a single or at most a few species. For example, *P. yoelii* is able to infect only rodents while *P.*

5 *falciparum* and *P. malariae* are able to infect humans. The parasitic infection to be targeted by the methods and compounds of the invention will depend upon the host species receiving the prophylactic treatment and the conditions to which that host will become exposed.

Parasites can be classified based on whether they are intracellular or extracellular. An "intracellular parasite" as used herein is a parasite whose entire life cycle is intracellular.

10 Examples of human intracellular parasites include *Leishmania* spp., *Plasmodium* spp., *Trypanosoma cruzi*, *Toxoplasma gondii*, *Babesia* spp., and *Trichinella spiralis*. An "extracellular parasite" as used herein is a parasite whose entire life cycle is extracellular. Extracellular parasites capable of infecting humans include *Entamoeba histolytica*, *Giardia lamblia*, *Enterocytozoon bieneusi*, *Naegleria* and *Acanthamoeba* as well as most helminths.

15 Yet another class of parasites is defined as being mainly extracellular but with an obligate intracellular existence at a critical stage in their life cycles. Such parasites are referred to herein as "obligate intracellular parasites". These parasites may exist most of their lives or only a small portion of their lives in an extracellular environment, but they all have at least one obligate intracellular stage in their life cycles. This latter category of parasites includes

20 *Trypanosoma rhodesiense* and *Trypanosoma gambiense*, *Isospora* spp., *Cryptosporidium* spp., *Eimeria* spp., *Neospora* spp., *Sarcocystis* spp., and *Schistosoma* spp. In one aspect, the invention relates to the prevention and treatment of infection resulting from intracellular parasites and obligate intracellular parasites which have at least in one stage of their life cycle that is intracellular. In some embodiments, the invention is directed to the prevention of

25 infection from obligate intracellular parasites which are predominantly intracellular. The methods of the invention are not expected to function in the prevention of infection by extracellular parasites, i.e., helminths. An exemplary and non-limiting list of parasites for some aspects of the invention is provided herein.

Blood-borne and/or tissues parasites include *Plasmodium* spp., *Babesia microti*,

30 *Babesia divergens*, *Leishmania tropica*, *Leishmania* spp., *Leishmania braziliensis*, *Leishmania donovani*, *Trypanosoma gambiense* and *Trypanosoma rhodesiense* (African

sleeping sickness), *Trypanosoma cruzi* (Chagas' disease), and *Toxoplasma gondii*.

Typical parasites infecting horses are *Gasterophilus* spp.; *Eimeria leuckarti*, *Giardia* spp.; *Tritrichomonas equi*; *Babesia* spp. (RBC's), *Theileria equi*; *Trypanosoma* spp.; *Klossiella equi*; *Sarcocystis* spp.

5 Typical parasites infecting swine include *Eimeria bebliecki*, *Eimeria scabra*, *Isospora suis*, *Giardia* spp.; *Balantidium coli*, *Entamoeba histolytica*; *Toxoplasma gondii* and *Sarcocystis* spp., and *Trichinella spiralis*.

The major parasites of dairy and beef cattle include *Eimeria* spp., *Cryptosporidium* sp., *Giardia* sp.; *Toxoplasma gondii*; *Babesia bovis* (RBC), *Babesia bigemina* (RBC),
10 *Trypanosoma* spp. (plasma), *Theileria* spp. (RBC); *Theileria parva* (lymphocytes); *Tritrichomonas foetus*; and *Sarcocystis* spp.

The major parasites of raptors include *Trichomonas gallinae*; *Coccidia* (*Eimeria* spp.); *Plasmodium relictum*, *Leucocytozoon danilewskyi* (owls), *Haemoproteus* spp., *Trypanosoma* spp.; *Histomonas*; *Cryptosporidium meleagridis*, *Cryptosporidium baileyi*, *Giardia*, *Eimeria*;
15 *Toxoplasma*.

Typical parasites infecting sheep and goats include *Eimeria* spp., *Cryptosporidium* sp., *Giardia* sp.; *Toxoplasma gondii*; *Babesia* spp. (RBC), *Trypanosoma* spp. (plasma), *Theileria* spp. (RBC); and *Sarcocystis* spp.

Typical parasitic infections in poultry include coccidiosis caused by *Eimeria*
20 *acervulina*, *E. necatrix*, *E. tenella*, *Isospora* spp. and *Eimeria truncata*; histomoniasis, caused by *Histomonas meleagridis* and *Histomonas gallinarum*; trichomoniasis caused by *Trichomonas gallinae*; and hexamitiasis caused by *Hexamita meleagridis*. Poultry can also be infected *Emeria maxima*, *Emeria meleagridis*, *Eimeria adenoeides*, *Eimeria meleagrimitis*, *Cryptosporidium*, *Eimeria brunetti*, *Emeria adenoeides*, *Leucocytozoon* spp., *Plasmodium*
25 spp., *Hemoproteus meleagridis*, *Toxoplasma gondii* and *Sarcocystis*.

Parasitic infections also pose serious problems in laboratory research settings involving animal colonies. Some examples of laboratory animals intended to be treated, or in which parasite infection is sought to be prevented, by the methods of the invention include mice, rats, rabbits, guinea pigs, nonhuman primates, as well as the aforementioned swine and
30 sheep.

Typical parasites in mice include *Leishmania* spp., *Plasmodium berghei*, *Plasmodium*

yoelii, *Giardia muris*, *Hexamita muris*; *Toxoplasma gondii*; *Trypanosoma duttoni* (plasma); *Klossiella muris*; *Sarcocystis* spp. Typical parasites in rats include *Giardia muris*, *Hexamita muris*; *Toxoplasma gondii*; *Trypanosoma lewisi* (plasma); *Trichinella spiralis*; *Sarcocystis* spp. Typical parasites in rabbits include *Eimeria* sp.; *Toxoplasma gondii*; *Nosema cuniculi*;
5 *Eimeria stiedae*, *Sarcocystis* spp. Typical parasites of the hamster include *Trichomonas* spp.; *Toxoplasma gondii*; *Trichinella spiralis*; *Sarcocystis* spp. Typical parasites in the guinea pig include *Balantidium caviae*; *Toxoplasma gondii*; *Klossiella caviae*; *Sarcocystis* spp.

The methods of the invention can also be applied to the treatment and/or prevention of parasitic infection in dogs, cats, birds, fish and ferrets. Typical parasites of birds include
10 *Trichomonas gallinae*; *Eimeria* spp., *Isospora* spp., *Giardia*; *Cryptosporidium*; *Sarcocystis* spp., *Toxoplasma gondii*, *Haemoproteus/Parahaemoproteus*, *Plasmodium* spp., *Leucocytozoon/Akiba*, *Atoxoplasma*, *Trypanosoma* spp. Typical parasites infecting dogs include *Trichinella spiralis*; *Isopora* spp., *Sarcocystis* spp., *Cryptosporidium* spp., *Hammondia* spp., *Giardia duodenalis (canis)*; *Balantidium coli*, *Entamoeba histolytica*;
15 *Hepatozoon canis*; *Toxoplasma gondii*, *Trypanosoma cruzi*; *Babesia canis*; *Leishmania amastigotes*; *Neospora caninum*.

Typical parasites infecting feline species include *Isospora* spp., *Toxoplasma gondii*, *Sarcocystis* spp., *Hammondia hammondi*, *Besnoitia* spp., *Giardia* spp.; *Entamoeba histolytica*; *Hepatozoon canis*, *Cytauxzoon* sp., *Cytauxzoon* sp., *Cytauxzoon* sp. (red cells, RE
20 cells).

Typical parasites infecting fish include *Hexamita* spp., *Eimeria* spp.; *Cryptobia* spp., *Nosema* spp., *Myxosoma* spp., *Chilodonella* spp., *Trichodina* spp.; *Plistophora* spp., *Myxosoma Henneguya*; *Costia* spp., *Ichthyophthirius* spp., and *Oodinium* spp.

Typical parasites of wild mammals include *Giardia* spp. (carnivores, herbivores),
25 *Isospora* spp. (carnivores), *Eimeria* spp. (carnivores, herbivores); *Theileria* spp. (herbivores), *Babesia* spp. (carnivores, herbivores), *Trypanosoma* spp. (carnivores, herbivores); *Schistosoma* spp. (herbivores); *Fasciola hepatica* (herbivores), *Fascioloides magna* (herbivores), *Fasciola gigantica* (herbivores), *Trichinella spiralis* (carnivores, herbivores).

Parasitic infections in zoos can also pose serious problems. Typical parasites of the
30 bovidae family (blesbok, antelope, banteng, eland, gaur, impala, klipspringer, kudu, gazelle) include *Eimeria* spp. Typical parasites in the pinnipedae family (seal, sea lion) include

Eimeria phocae. Typical parasites in the camelidae family (camels, llamas) include *Eimeria* spp. Typical parasites of the giraffidae family (giraffes) include *Eimeria* spp. Typical parasites in the elephantidae family (African and Asian) include *Fasciola* spp. Typical parasites of lower primates (chimpanzees, orangutans, apes, baboons, macaques, monkeys) include *Giardia* sp.; *Balantidium coli*, *Entamoeba histolytica*, *Sarcocystis* spp., *Toxoplasma gondii*; *Plasmodium* spp. (RBC), *Babesia* spp. (RBC), *Trypanosoma* spp. (plasma), *Leishmania* spp. (macrophages).

Diseases caused by parasites can be acute, as in the case of malaria (*Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae*) or AIDS-related opportunistic pathogenic infection (*Toxoplasma* and *Cryptosporidium*), or chronic, as with heart disease in South America (*Trypanosoma cruzi*), fluke-like disease (schistosomiasis) and blindness (*Onchocerca volvulus*) in humans. Parasite-related diseases also include: in cattle, ostertagiasis caused by *Ostertagia* infection and manifest as diarrhea, anorexia or loss of appetite and weight loss; in sheep, haemonchosis caused by *H. contortus* infection and manifest as unexpected death, weakness, anemia, hypoproteinemia, subcutaneous edema, weight loss, or poor or no weight gain.

According to some aspects of the invention, the subject is free of parasitic infection and disease related symptoms. In some instances, subjects have been put at risk of contracting a parasite through close contact with an identified or suspected carrier or the bodily waste of such an individual. In another aspect, the invention is directed to a method for treating a subject who has developed a parasitic infection as a result of being in contact with an infectious parasite such as from an individual having a parasitic infection. Parasitic infection in a subject is often associated with a number of symptoms or conditions, combinations of which may be used in some embodiments to identify the particular parasite involved. Many of the individual symptoms are shared by a number of parasitic infections and include malaise, lethargy, fatigue, headache, fever, chills, weakness, fast heartbeat, heart pain, blurry or unclear vision, anemia, loss of appetite, weight loss or failure of weight gain, lower abdominal or back pain ranging from diffuse to severe, diarrhea, numb hands, sexual dysfunction (in male subjects), menstrual irregularity, jaundiced skin colour and itchy orifices including ears, nose and anus. Severe malaria can manifest itself in unarousable coma (cerebral malaria), renal failure, severe anemia, pulmonary edema, hypoglycemia,

hypotension or shock, bleeding or disseminated intravascular coagulation, convulsions, acidemia or acidosis, hemoglobinuria, jaundice and hyperpyrexia. Symptoms particularly associated with gastrointestinal parasitic infections also include loss of blood resulting in pale mucous membranes, diarrhea with loss of water and electrolyte disturbances, poor weight gains or even weight loss in severe infections, protein losses, hypoproteinemia and associated edema, anorexia and reduced food intake, anemia, reduced digestion and absorption.

Diagnosis of a parasite infection in non-human animals can involve the initial observance of symptoms associated with particular infections. For example, haemonchosis in sheep should be suspected if the following conditions are observed: unexpected deaths, weakness, anemia, hypoproteinemia, subcutaneous edema, poor weight gains or weight loss. These conditions will be apparent and well known to a veterinarian.

The diagnosis of a parasitic infection in an individual can be used to determine the need for prophylactic treatment in other subjects previously in contact or likely to be in contact with the afflicted individual using the methods of the invention as well as for treatment of the infected individual. A number of laboratory tests for the diagnosis of parasitic infections, well known in the art, are described, for example, in Harrison's: Principles of Internal Medicine (McGraw Hill, Inc., New York).

Methods for diagnosing parasitic infections are generally similar for human and non-human parasitic infections. Procedures for diagnosing parasitism vary depending on the type of parasite to be detected. These procedures are well known to any clinician or veterinarian and can be easily performed in almost any clinical or veterinary practice. Macroscopic and microscopic examination of a bodily sample is usually initially performed to detect the presence of ova and adult parasites. Tissue parasites can sometimes be detected through the examination of biopsies and aspirates. A bodily sample can be a liquid such as urine, saliva, cerebrospinal fluid, blood, serum, bronchoalveolar lavage, sputum, bile or the like; a solid or semi-solid such as tissues, feces, or the like; or, alternatively, a solid tissue such as those commonly used in histological diagnosis.

Tests for parasites in agricultural livestock include direct smear of bodily liquid such as blood or bodily waste such as feces; fecal flotation fluids, centrifugation technique with flotation fluid (magnesium sulfate), modified Wisconsin Procedure for egg counts by a flotation method (for cattle, horses, dogs, cats and swine), modified Knott's Method of

concentrating microfilaria, skin scraping and squash preparation for the diagnosis of trichinosis. Generally liquid samples should be stained in order to better visualize any parasite bodies. Giemsa or Wright's stain are appropriate for analysis of a number of parasites including *Plasmodium* spp., *Leishmania* spp., African trypanosomes, *Trypanosoma cruzi*,
5 *Toxoplasma gondii* and *Naegleria fowleri* in the blood, urine or spinal fluid.

A diagnosis of coccidiosis in poultry can be established by preparing a wet mount of a mucosal scraping from the intestines of an infected bird and examining it by light microscopy. The coccidial oocytes and schizonts can readily be identified at 100X magnification. A fecal flotation is also very effective in demonstrating coccidial oocysts. Histomoniasis can be
10 diagnosed based on characteristic gross lesions and/or histologic lesions and *H. gallinarum* can be isolated from tissues of freshly killed affected birds in special broth media.

Parasites can be detected in humans by a number of well recognized specific binding assays which have been previously discussed in U.S. Patent Nos. 4,366,241; 4,376,110; 4,517,288 and 4,837,168, all of which are incorporated in their entirety by reference herein.

15 Other laboratory tests available to the clinician or veterinarian include immunodetection methods for the detection of parasite antigens or host antiparasite antibodies. For example, enzyme-linked immunosorbent assay (ELISA) can be employed in the detection of tissue parasites such as *Toxoplasma*. Still more sensitive assays involve nucleic acid amplification methods such as polymerase chain reaction (PCR) and nucleic acid hybridization methods for
20 detecting parasitic nucleic acids. Detection of parasitic infection through nucleic acid based methods usually leads to the diagnosis of low grade malaria not otherwise detectable using standard clinical and laboratory techniques. The levels of parasite antigens or nucleic acids can be compared to the levels in a control biological fluid or tissue sample taken from an uninfected individual. A positively identified subject would then signal the required
25 therapeutic treatment in that individual and also the prophylactic treatment of other individuals. A person of ordinary skill in the art could easily apply any of the foregoing tests to determine when a subject has a parasitic infection in order to plan a treatment course for that individual, for individuals previously in contact with that individual and for individuals expected to be in contact with that individual prior to the elimination of the parasitic infection
30 by the methods of the invention. These diagnostic tests can also be used to determine the relative efficacy of the prophylactic and treatment modes of the invention, particularly with

respect to administered dosages of the CpG oligonucleotides.

The CpG oligonucleotides of the invention are nucleic acid molecules which contain an unmethylated cytosine-guanine dinucleotide sequence (i.e. "CpG DNA" or DNA containing a 5' cytosine followed by 3' guanosine and linked by a phosphate bond) and
5 activate the immune system. The CpG oligonucleotides can be double-stranded or single-stranded. Generally, although double-stranded molecules are more stable *in vivo*, single-stranded molecules have increased immune activity.

The terms "nucleic acid" and "oligonucleotide" are used interchangeably to mean multiple nucleotides (i.e. molecules comprising a sugar (e.g. ribose or deoxyribose) linked to a
10 phosphate group and to an exchangeable organic base, which is either a substituted pyrimidine (e.g. cytosine (C), thymine (T) or uracil (U)) or a substituted purine (e.g. adenine (A) or guanine (G)). As used herein, the terms refer to oligoribonucleotides as well as oligodeoxyribonucleotides. The terms shall also include polynucleosides (i.e. a polynucleotide minus the phosphate) and any other organic base containing polymer. Nucleic
15 acid molecules can be obtained from existing nucleic acid sources (e.g. genomic or cDNA), but are preferably synthetic (e.g. produced by oligonucleotide synthesis). The entire CpG oligonucleotide can be unmethylated or portions may be unmethylated but at least the C of the 5' CG 3' must be unmethylated.

The CpG oligonucleotide has a sequence including at least the following formula:



In one preferred embodiment the invention provides a CpG oligonucleotide represented by at least the formula:



wherein at least one nucleotide separates consecutive CpGs; X_1 is adenine, guanine, or
25 thymine; X_2 is cytosine, adenine, or thymine; N is any nucleotide and N_1 and N_2 are nucleic acid sequences composed of from about 0-25 N's each.

In another embodiment the invention provides an isolated CpG oligonucleotide represented by at least the formula:



wherein at least one nucleotide separates consecutive CpGs; X_1X_2 is selected from the group

consisting of GpT, GpG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT, and TpG; X_3X_4 is selected from the group consisting of TpT, CpT, ApT, TpG, ApG, CpG, TpC, ApC, CpC, TpA, ApA, and CpA; N is any nucleotide and N_1 and N_2 are nucleic acid sequences composed of from about 0-25 N's each. In a preferred embodiment N_1 and N_2 of the nucleic acid do not
5 contain a CCGG or a CGCG quadmer or more than one CCG or CGG trimer especially if the oligonucleotide has a modified phosphate backbone. In another preferred embodiment the CpG oligonucleotide has the sequence 5'TCN₁TX₁X₂CGX₃X₄3'.

Preferably the CpG oligonucleotides of the invention include X_1X_2 selected from the group consisting of GpT, GpG, GpA and ApA and X_3X_4 is selected from the group consisting
10 of TpT and CpT. For facilitating uptake into cells, CpG containing oligonucleotides are preferably in the range of 6 to 30 bases in length. However, nucleic acids of any size greater than 6 nucleotides (even many kb long) are capable of inducing an immune response according to the invention if sufficient immunostimulatory motifs are present and if the CpG oligonucleotide enters the cell, since larger nucleic acids are degraded into oligonucleotides
15 inside of cells. Preferred synthetic oligonucleotides do not include a CCGG quadmer or more than one CCG or CGG trimer at or near the 5' and/or 3' terminals. Stabilized oligonucleotides, where the oligonucleotide incorporates a phosphate backbone modification, as discussed in more detail below are also preferred. The modification may be, for example, a phosphorothioate or phosphorodithioate modification. Preferably, the phosphate backbone
20 modification occurs in the region 5' to the CpG of the nucleic acid for example, at the first two nucleotides of the 5' end of the oligonucleotide. Further, the phosphate backbone modification may occur in the region 3' to the CpG of the nucleic acid for example, at the last five nucleotides of the 3' end of the nucleic acid. Alternatively the oligonucleotide may be completely or partially modified (referred to herein as a chimeric phosphate modification).
25 It should be noted however that the kinetics of biological activity with different CpG oligonucleotides may vary depending upon whether a phosphorothioate, a phosphodiester, or a chimeric backbone is employed. For example, CpG oligonucleotides represented as SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:55 and SEQ ID NO:61 may have higher activity if presented in a phosphodiester or a chimeric
30 backbone, rather than an exclusively phosphorothioate backbone. Alternatively, they may have the same activity but the kinetics may vary. The appropriate backbone for each sequence

may be identified by those of ordinary skill in the art without undue experimentation.

Preferably the CpG oligonucleotide is in the range of between 6 and 100 and more preferably between 8 and 30 nucleotides in size. Alternatively, CpG oligonucleotides can be produced on a large scale in plasmids and degraded into oligonucleotides.

5 "Palindromic sequence" shall mean an inverted repeat (i.e. a sequence such as ABCDEE'D'C'B'A' in which A and A' are bases capable of forming the usual Watson-Crick base pairs. *In vivo*, such sequences may form double-stranded structures. In one embodiment the CpG oligonucleotide contains a palindromic sequence. A palindromic sequence used in this context refers to a palindrome in which the CpG is part of the palindrome, and preferably
10 is the center of the palindrome. In another embodiment the CpG oligonucleotide is free of a palindrome. A CpG oligonucleotide that is free of a palindrome is one in which the CpG dinucleotide is not part of a palindrome. Such an oligonucleotide may include a palindrome in which the CpG is not part of the palindrome.

A "stabilized nucleic acid molecule" shall mean a nucleic acid molecule that is
15 relatively resistant to *in vivo* degradation (e.g. via an exo- or endo-nuclease). Stabilization can be a function of length or secondary structure. Unmethylated CpG oligonucleotides that are tens to hundreds of kbs long are relatively resistant to *in vivo* degradation. For shorter CpG oligonucleotides, secondary structure can stabilize and increase their effect. For example, if the 3' end of an oligonucleotide has self-complementarity to an upstream region, so that it can
20 fold back and form a sort of stem loop structure, then the oligonucleotide becomes stabilized and therefore exhibits more activity.

Preferred stabilized CpG oligonucleotides of the instant invention have a modified backbone. It has been demonstrated that modification of the oligonucleotide backbone provides enhanced activity of the CpG oligonucleotides when administered *in vivo*. CpG
25 constructs, including at least two phosphorothioate linkages at the 5' end of the oligonucleotide and multiple, preferably 5, phosphorothioate linkages at the 3' end provides maximal activity and protects the oligonucleotide from degradation by intracellular exo- and endo-nucleases. Other modified CpG oligonucleotides include phosphodiester modified oligonucleotide, combinations of phosphodiester and phosphorothioate oligonucleotide,
30 methylphosphonate, methylphosphorothioate, phosphorodithioate, and combinations thereof. Each of these combinations and their particular effects on immune cells is discussed in more

detail in copending PCT Published Patent Applications claiming priority to U.S. Serial Nos. 08/738,652 and 08/960,774, filed on October 30, 1996 and October 30, 1997 respectively, the entire contents of which is hereby incorporated by reference. It is believed that these modified CpG oligonucleotides may show more stimulatory activity due to enhanced nuclease
5 resistance, increased cellular uptake, increased protein binding, and/or altered intracellular localization.

Both phosphorothioate and phosphodiester oligonucleotides containing CpG motifs are active in immune cells. However, based on the concentration needed to induce CpG specific effects, the nuclease resistant phosphorothioate backbone CpG oligonucleotides are
10 more potent (0.4 μ g/ml for the phosphorothioate vs. a total of 40 μ g/ml for phosphodiester).

Other stabilized oligonucleotides include: nonionic DNA analogs, such as alkyl- and aryl-phosphates (in which the charged phosphonate oxygen is replaced by an alkyl or aryl group), phosphodiester and alkylphosphotriesters, in which the charged oxygen moiety is alkylated. Oligonucleotides which contain diol, such as tetraethyleneglycol or
15 hexaethyleneglycol, at either or both termini have also been shown to be substantially resistant to nuclease degradation.

The nucleic acid sequences of the invention which are useful for preventing parasitic infection are those broadly described above and disclosed in PCT Published Patent Applications claiming priority to U.S. Serial Nos. 08/738,652 and 08/960,774, filed on
20 October 30, 1996 and October 30, 1997 respectively. Exemplary sequences include but are not limited to those set forth in Table 1.

Table 1 -sequences

| | | |
|----|----------------------|-----------------|
| | GCTAGACGTTAGCGT | (SEQ ID NO: 1) |
| | GCTAGATGTTAGCGT | (SEQ ID NO: 2) |
| 25 | GCTAGACGTTAGZGT | (SEQ ID NO: 3) |
| | GCATGACGTTGAGCT | (SEQ ID NO: 4) |
| | ATGGAAGGTCCAGCGTTCTC | (SEQ ID NO: 5) |
| | ATCGACTCTCGAGCGTTCTC | (SEQ ID NO: 6) |
| | ATZGACTCTCGAGCGTTCTC | (SEQ ID NO: 7) |
| 30 | ATCGACTCTCGAGCGTTZTC | (SEQ ID NO: 8) |
| | ATCGACTCTCGAACGTTCTC | (SEQ ID NO: 9) |
| | GAGAACGCTGGACCTTCCAT | (SEQ ID NO: 10) |
| | GAGAACGCTCGACCTTCCAT | (SEQ ID NO: 11) |
| | GAGAACGCTCGACCTTCGAT | (SEQ ID NO: 12) |
| 35 | GAGCAAGCTGGACCTTCCAT | (SEQ ID NO: 13) |

| | | |
|----|---------------------------|-----------------|
| | GAGAACGCTGGACZTTCCAT | (SEQ ID NO: 14) |
| | GAGAACGATGGACCTTCCAT | (SEQ ID NO: 15) |
| | GAGAACGCTCCAGCACTGAT | (SEQ ID NO: 16) |
| | CCATGTCGGTCCTGATGCT | (SEQ ID NO: 17) |
| 5 | TCCATGTCGGTZCTGATGCT | (SEQ ID NO: 18) |
| | TCCATGACGTTCTGATGCT | (SEQ ID NO: 19) |
| | TCCATGTCGGTCCTGACGCA | (SEQ ID NO: 20) |
| | TCAACGTT | (SEQ ID NO: 21) |
| | TCAGCGCT | (SEQ ID NO: 22) |
| 10 | TCTTCGAT | (SEQ ID NO: 23) |
| | TCTTCGAA | (SEQ ID NO: 24) |
| | CAACGTT | (SEQ ID NO: 25) |
| | CCAACGTT | (SEQ ID NO: 26) |
| | CAACGTTCT | (SEQ ID NO: 27) |
| 15 | TCAACGTC | (SEQ ID NO: 28) |
| | ATGGACTCTCCAGCGTTCTC | (SEQ ID NO: 29) |
| | ATAGGAGGTCCAACGTTCTC | (SEQ ID NO: 30) |
| | ATCGACTCTCGAGCGTTCTC | (SEQ ID NO: 31) |
| | ATGGAGGCTCCATCGTTCTC | (SEQ ID NO: 32) |
| 20 | ATCGACTCTCGAGZGTTCTC | (SEQ ID NO: 33) |
| | GCATGACGTTGAGCT | (SEQ ID NO: 34) |
| | TCCATGTCGGTCCTGATGCT | (SEQ ID NO: 35) |
| | TCCATGGCGGTCCTGATGCT | (SEQ ID NO: 36) |
| | TCCATGACGGTCCTGATGCT | (SEQ ID NO: 37) |
| 25 | TCCATGTGATCCTGATGCT | (SEQ ID NO: 38) |
| | TCCATGTGCTCCTGATGCT | (SEQ ID NO: 39) |
| | TCCATGTGTTCTGATGCT | (SEQ ID NO: 40) |
| | TCCATAACGTTCTGATGCT | (SEQ ID NO: 41) |
| | TCCATGACGTCCCTGATGCT | (SEQ ID NO: 42) |
| 30 | TCCATCACGTGCCTGATGCT | (SEQ ID NO: 43) |
| | GGGGTCAACGTTGACGGGG | (SEQ ID NO: 44) |
| | GGGGTCAGTCGTGACGGGG | (SEQ ID NO: 45) |
| | GCTAGACGTTAGTGT | (SEQ ID NO: 46) |
| | TCCATGTGTTCTGATGCT | (SEQ ID NO: 47) |
| 35 | ACCATGGACGATCTGTTTCCCCTC | (SEQ ID NO: 48) |
| | TCTCCCAGCGTGCGCCAT | (SEQ ID NO: 49) |
| | TACCGCGTGCGACCCTCT | (SEQ ID NO: 50) |
| | ACCATGGACGAACTGTTTCCCCTC | (SEQ ID NO: 51) |
| | ACCATGGACGAGCTGTTTCCCCTC | (SEQ ID NO: 52) |
| 40 | ACCATGGACGACCTGTTTCCCCTC | (SEQ ID NO: 53) |
| | ACCATGGACGTA CTGTTTCCCCTC | (SEQ ID NO: 54) |
| | ACCATGGACGGTCTGTTTCCCCTC | (SEQ ID NO: 55) |
| | ACCATGGACGTTCTGTTTCCCCTC | (SEQ ID NO: 56) |
| | CACGTTGAGGGGCAT | (SEQ ID NO: 57) |
| 45 | TCAGCGTGCGCC | (SEQ ID NO: 58) |
| | ATGACGTTCTGACGTT | (SEQ ID NO: 59) |

| | | |
|----|--|-----------------|
| | TCTCCCAGCGGGCGCAT | (SEQ ID NO: 60) |
| | TCTCCCAGCGCGGCCAT | (SEQ ID NO: 61) |
| | TCCATGTCGTTCTCTGTCGTT | (SEQ ID NO: 62) |
| | TCCATAGCGTTCCTAGCGTT | (SEQ ID NO: 63) |
| 5 | TCGTCGCTGTCTCCGCTTCTT | (SEQ ID NO: 64) |
| | TCCTGACGTTCCCTGACGTT | (SEQ ID NO: 65) |
| | TCCTGT <u>CGT</u> TCCTGT <u>CGT</u> T | (SEQ ID NO: 66) |
| | TCCATGT <u>CGT</u> TTTTGT <u>CGT</u> T | (SEQ ID NO: 67) |
| | TCCTGT <u>CGT</u> TCCTGT <u>CGT</u> T | (SEQ ID NO: 68) |
| 10 | TCCTGT <u>CGT</u> TCCTGT <u>CGT</u> T | (SEQ ID NO: 69) |
| | TCCTGT <u>CGT</u> TTTTGT <u>CGT</u> T | (SEQ ID NO: 70) |
| | <u>TCGT</u> <u>CGT</u> GTCTGCCCTTCTT | (SEQ ID NO: 71) |
| | <u>TCGT</u> <u>CGT</u> GTGT <u>CGT</u> TTCTT | (SEQ ID NO: 72) |
| | TCCATG <u>CGT</u> G <u>CGT</u> G <u>CGT</u> TTT | (SEQ ID NO: 73) |
| 15 | TCCATG <u>CGT</u> TG <u>CGT</u> TG <u>CGT</u> T | (SEQ ID NO: 74) |
| | TCCACGACGTTTTTCGACGTT | (SEQ ID NO: 75) |
| | <u>TCGT</u> <u>CGT</u> TGTCGTTGT <u>CGT</u> T | (SEQ ID NO: 76) |
| | <u>TCGT</u> <u>CGT</u> TTTGT <u>CGT</u> TTTGT <u>CGT</u> T | (SEQ ID NO: 77) |
| | <u>TCGT</u> <u>CGT</u> TGTCGTTTGT <u>CGT</u> T | (SEQ ID NO: 78) |
| 20 | G <u>CGT</u> G <u>CGT</u> TGTCGTTGT <u>CGT</u> T | (SEQ ID NO: 79) |
| | TGT <u>CGT</u> TTGT <u>CGT</u> TTGT <u>CGT</u> T | (SEQ ID NO: 80) |
| | TGT <u>CGT</u> TGTCGTTGT <u>CGT</u> TGTCGTT | (SEQ ID NO: 81) |
| | TGT <u>CGT</u> TGTCGTTGT <u>CGT</u> T | (SEQ ID NO: 82) |
| | <u>TCGT</u> <u>CGT</u> <u>CGT</u> <u>CGT</u> T | (SEQ ID NO: 83) |
| 25 | TGT <u>CGT</u> TGTCGTT | (SEQ ID NO: 84) |
| | TCCATAG <u>CGT</u> TCTCTAG <u>CGT</u> T | (SEQ ID NO: 85) |
| | TCCATGACGTTCTCTGACGTT | (SEQ ID NO: 86) |
| | GTCGTT | (SEQ ID NO: 87) |
| | TGTCGTT | (SEQ ID NO: 88) |
| 30 | TCTCCCAG <u>CGT</u> G <u>CGC</u> CCAT | (SEQ ID NO: 89) |
| | GTCGCT | (SEQ ID NO: 90) |
| | TGTCGCT | (SEQ ID NO: 91) |

For use in the instant invention, nucleic acids can be synthesized *de novo* using any of

35 a number of procedures well known in the art. Such nucleic acids are referred to as "synthetic oligonucleotides". For example, the b-cyanoethyl phosphoramidite method (S.L. Beaucage and M.H. Caruthers, 1981, *Tet. Let.* 22:1859); nucleoside H-phosphonate method (Garegg, et al., 1986, *Tet. Let.* 27:4051-4051; Froehler, et al., 1986, *Nucl. Acid. Res.* 14:5399-5407; Garegg, et al., 1986, *Tet. Let.* 27:4055-4058, Gaffney, et al., 1988), *Tet. Let.* 29:2619-2622.

40 These chemistries can be performed by a variety of automated oligonucleotide synthesizers available in the market. Alternatively, oligonucleotides can be prepared from existing nucleic

acid sequences (e.g. genomic or cDNA) using known techniques, such as those employing restriction enzymes, exonucleases or endonucleases. Such oligonucleotides are referred to as isolated oligonucleotides.

For use *in vivo*, nucleic acids are preferably relatively resistant to degradation (e.g. via
5 endo- and exo-nucleases). Secondary structures, such as stem loops, can stabilize nucleic acids against degradation. Alternatively, nucleic acid stabilization can be accomplished via phosphate backbone modifications as discussed above. A preferred stabilized nucleic acid can be accomplished via phosphate backbone modifications. A preferred stabilized nucleic acid has at least a partial phosphorothioate modified backbone. Phosphorothioates may be
10 synthesized using automated techniques employing either phosphoramidate or H-phosphonate chemistries. Aryl- and alkyl-phosphonates can be made for example as described in U.S. Patent No. 4,469,863; and alkylphosphotriesters (in which the charged oxygen moiety is alkylated as described in U.S. Patent No. 5,023,243 and European Patent No. 092,574) can be prepared by automated solid phase synthesis using commercially available reagents. Methods
15 for making other DNA backbone modifications and substitutions have been described (Uhlmann, E. and Peyman, A., 1990, *Chem Rev.* 90:544; Goodchild, J., 1990, *Bioconjugate Chem.* 1:165). 2'-O-methyl nucleic acids with CpG motifs can also cause detectable immune activation, as do ethoxy-modified CpG nucleic acids. In fact, no backbone modifications have been found that completely abolish the immune response, although it is greatly reduced by
20 replacing the C with a 5-methyl C.

In one aspect, the methods of the invention involve administering to a subject, prior to parasite exposure, a CpG containing oligonucleotide in an amount effective to prevent a parasitic infection in the subject. Prior administration of a CpG oligonucleotide greatly benefits the subject by inducing a response within the subject consisting at least of an
25 activated innate immune system response prior to, during or following the exposure to a parasite. By "prior administration" it is meant that administration occurs before exposure to the parasite. In some embodiments, the compounds of the invention may be administered with a greater than 60 day period of time between the administration and the parasite exposure. In other embodiments, the CpG oligonucleotides may be administered at least 50,
30 or 40, or 30, or 14, or 7 days prior to parasite exposure. In yet other embodiments, the CpG oligonucleotides may be administered within a 7, 6, 5, 4, 3 or 2 day period prior to infection.

In still other embodiments, the CpG oligonucleotide of the invention may be administered at least 24 hours prior to suspected parasitic exposure. And in still further embodiments, the CpG oligonucleotides may be administered within 24, 12 or 4 hours of parasite infection. Timing will depend upon the particular parasite infection to be treated and/or prevented as well as the mode of delivery (i.e., whether acute or chronic release required). If chronic delivery or treatment is required, then, in some embodiments, CpG oligonucleotides may be administered with a greater than 7 day period between the CpG oligonucleotide administration and the parasite exposure. In such cases, higher doses may be used but are not always required. In preferred embodiments, the CpG oligonucleotides are administered within 2 days of parasite exposure. The period of protection will depend upon the dose of CpG oligonucleotide administered, thus high doses can provide longer lasting protection. The length of protection will also depend upon the mode of administration and the particular infection being prevented. Administration may also be repeated, such that a more prolonged anti-parasitic effect can be obtained following multiple treatments with CpG oligonucleotides or delivery of CpG oligonucleotides in controlled release vesicles (e.g., micro encapsulated) or formulated in such a way to retard *in vivo* degradation (e.g., liposomes).

In another aspect, the invention relates to the treatment of subjects infected with a parasite. In preferred embodiments the subject has been exposed and is currently suffering from an infection by the following parasites: *Plasmodium* spp., *Babesia* spp., *Trypanosoma cruzi*, *Toxoplasma gondii* and *Trichinella spiralis*. In these embodiments, the CpG oligonucleotides are effective in treating the infection even if administered after exposure to the parasite. The compounds of the invention may be administered immediately after the parasite exposure or after a period of time. For example, the CpG oligonucleotides may be administered once the parasitic infection has been diagnosed which may range from a few days to several weeks after parasite exposure or contact. In some embodiments, the CpG oligonucleotides may be administered within 24 hours or 48 hours after parasite infection (i.e., parasite exposure). If diagnosis or treatment is delayed, it is also envisioned that the oligonucleotides may be administered within 7 days of infection. There may still be other situations in which even longer (i.e., greater than 7 days, 14 days or 30 days) period of time may elapse between parasite exposure and oligonucleotide administration.

Although not wishing to be bound by any particular theory, unmethylated CpG

oligonucleotides may exert parasitic protection at least in part through the induction of an innate non-antigen specific immune response via the activation of immune cells including antigen presenting cells such as macrophages and dendritic cells, natural killer (NK) cells and granulocytes such as neutrophils and basophils. CpG oligonucleotides may also function by inducing a cell-mediated immune response through the activation of Th1 cells and the induction of Th1 cytokines, such as IL-12 and IFN- γ . Evidence that the anti-parasitic effect induced by CpG oligonucleotides is at least in part cytokine mediated is the observation that such protection can be completely or largely abrogated by treatment with monoclonal antibodies against IL-12 or anti-IFN- γ . Previously it had been known that administration of IL-12 could combat parasitic infection. Parenteral injection of recombinant IL-12 into mice (Sedegah *et al.*, 1994) or monkeys (Hoffman *et al.*, 1997) two days before malaria sporozoite challenge completely prevented development of blood stage infection. However, it was not known prior to the present discovery that administration of CpG containing oligonucleotides could induce sufficient levels of endogenous IL-12 to mount an immune response. This discovery and the ensuing invention thus provide benefit over the sole administration of IL-12 particularly since administration of IL-12, besides being costly, can cause cytokine imbalance in a subject. This imbalance is less likely to occur if the IL-12 induction occurs naturally as a result of mimicing a pathogenic invasion via the administration of unmethylated CpG containing oligonucleotides. In the latter instance, the body mounts an orchestrated response involving the cooperation of several humoural factors and cell types. It was also previously shown that administration of anti-IFN- γ to the mice eliminated the IL-12 protective effect and that the protection in the monkeys was associated with circulating levels of IFN- γ . Thus, it is presumed that administration of recombinant IL-12 enhances the production of IFN- γ , which in turn induces the intracellular generation of nitric oxide leading to the destruction of the infected hepatocytes. This model is supported by *in vitro* observations that hepatocytes infected with *P. yoelii* and *P. falciparum* can be eliminated by exposure to IFN- γ (Ferreira *et al.*, 1986, Mallouk *et al.*, 1991, 1994) and that this activity is prevented by inhibition of iNOS (Mellouk *et al.*, 1994). Again although not intending to be bound by any particular theory, it is possible that IL-12 plays a role in the subsequent induction of IFN- γ which in turn induces the synthesis of nitric oxide (NO). NO ultimately plays a role in the elimination of infected cells including malaria diseased hepatocytes.

Thus in one aspect, the invention relates to the IL-12 and IFN related immune response induced by CpG oligonucleotides. In still another aspect, the invention is not limited to IL-12 and/or IFN immune mechanisms, but rather can encompass each and every immune and non-immune mechanism by which CpG oligonucleotides exert protection or therapeutic benefit against parasite infection and associated disease.

The CpG oligonucleotides of the invention are administered in effective amounts. An effective amount is a dosage of CpG oligonucleotides sufficient to provide a medically desirable result. In one aspect of the invention, the CpG oligonucleotides are administered in effective amounts to prevent a parasitic infection or to protect a subject from a parasitic infection. In still another aspect of the invention, the CpG oligonucleotides are administered in effective amounts to reduce the level of, inhibit or eliminate a parasitic infection in a subject having a parasitic infection. The presence of a parasitic infection can be determined using routine assays known in the art as described above. It can be assessed, for example, by an absence of parasite bodies, fragments or ova in at least three consecutive tissue, fluid or waste samplings. Alternatively, if the assay method used for detecting parasites in the body comprises an immunodetection or nucleic acid amplification and/or hybridization method, then a conclusive negative finding may still require a number of consecutive negative findings over a period of a week rather than a single negative finding. The effective amount will vary with the particular condition being treated, the age and physical condition of the subject being treated, the severity of the condition, the duration of the treatment, the nature of the concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. The effective amount may also vary depending on whether a prophylactic effect or a change in the course of an infection is desired. For example, in connection with liver affected malaria (i.e., *Plasmodium* infection), an effective amount is that amount which prevents the appearance of, reduces the level of, or eliminates malaria infected hepatocytes and erythrocytes, or leads to the absence of *Plasmodium* parasites in the blood stream or in tissues of interest such as the liver. Likewise, an effective amount for preventing a parasite infection related disease is an amount sufficient to prevent the symptoms of such diseases as described herein. If the subject to be treated is already infected with a parasite, then an effective amount may be that amount necessary to reduce the number or, in some instances, eliminate altogether parasite bodies and/or fragments in a

sample of bodily fluid, tissue or waste. In other embodiments, an effective amount may be that amount necessary to reduce or eliminate the number and severity of symptoms relating to the particular parasite infection in a subject having a parasitic infection. An effective amount, is not however, an amount which is toxic to the subject. Thus, it will be understood that the
5 CpG oligonucleotides of the invention can be used to treat the above-noted conditions prophylactically in subjects at risk of developing the foregoing conditions, or therapeutically in subjects known or suspected to have a parasitic infection.

Generally, an effective amount will vary with the subject's age, condition, and sex, as well as the nature and extent of the parasitic infection being prevented in the subject, all of
10 which can be determined by one of ordinary skill in the art. The dosage may be adjusted by the individual physician or veterinarian, particularly in the event of any complication. A prophylactically effective amount typically varies from 0.01 mg/kg to about 1000 mg/kg, preferably from about 0.1 mg/kg to about 200 mg/kg, and most preferably from about 0.2 mg/kg to about 20 mg/kg, in one or more dose administrations daily, every few days or
15 weekly. Additionally, the dose to be administered will depend upon the timing and mode of administration. For example, if a single dose is administered or if a long interval of time is anticipated between dose administration and parasite exposure, then a higher dose may be necessary. Alternatively, if a dose is slowly infused, the amount delivered in an individual dose may also be higher than would be otherwise tolerated.

20 One of skill in the art can determine an effective amount of a CpG oligonucleotides using *in vitro* or *in vivo* screening assays. For example, one screening assay useful according to the invention measures the ability of the CpG oligonucleotide to lyse malaria infected hepatocytes in vitro. The prevention of parasitemia following parasite challenge may also be assessed in an *in vivo* model. An exemplary assay for measuring the ability of a CpG
25 oligonucleotide of the invention to protect mice from *Plasmodium* infection is provided in the Examples. The assay measures the ability of CpG oligonucleotides to prevent active infection following inoculation of *Plasmodium* sporozoites. Results of assays similar to those described in the Examples can be used to select CpG oligonucleotides and doses of such for prophylactic applications. The CpG oligonucleotides useful in the invention preferably
30 exhibit a complete inhibition of parasite infection as measured by a number of clinical, laboratory or molecular assays (as described herein). *In vivo* assays similar to those described

above can be used to identify CpG oligonucleotides and doses thereof which are useful in the treatment of an established parasite infection. For example, following exposure to Plasmodium sporozoities, mice are administered CpG oligonucleotides at various doses either in a single or multiple boli. At a set time after CpG oligonucleotide administration, the
5 animals and/or their waste are analyzed for the presence of parasites. In the case of blood borne parasites such as Plasmodium, a stained blood smear may suffice to detect the presence of parasites. In still other assays when the effect of CpG oligonucleotides on tissue parasites is being tested it may be necessary to sacrifice the animal in order to harvest the infected tissue. Subsequent detection methods for the infected tissue may include immunodetection
10 methods such as ELISA or nucleic acid based methods such as PCR. It should be apparent that using no more than routine skill in the art, it is possible to identify alternative CpG oligonucleotides which prevent and/or treat parasite infection and determine the appropriate range of use.

The compositions of the invention can be administered alone or in combination with
15 one or more non-CpG oligonucleotide therapeutic agents. Non-CpG oligonucleotide therapeutic agents, for the purpose of the invention, are agents useful in the prevention and treatment of parasitic infection and subsequent disease which are not CpG oligonucleotides as defined herein. Non-CpG oligonucleotide therapeutic agents may be administered together or at a timed interval from the administration of the CpG oligonucleotides. In embodiments
20 which seek to induce an immune response, a useful non-CpG oligonucleotide therapeutic agent is an immune response inducer such as a cytokine or antibody. Non-CpG oligonucleotide therapeutic agents can also include parasiticide agents previously used in the treatment of parasitic infection. Parasiticides are well known in the art and generally commercially available. Coadministration of the CpG oligonucleotides with other non-CpG
25 oligonucleotide prophylactic or therapeutic agents is also possible.

Coadministered compounds may be those known to be active against a particular parasitic disease. Examples of parasiticides useful for human administration include but are not limited to albendazole, amphotericin B, benznidazole, bithionol, chloroquine HCl, chloroquine phosphate, clindamycin, dehydroemetine, diethylcarbamazine, diloxanide furoate,
30 eflornithine, furazolidone, glucocorticoids, halofantrine, iodoquinol, ivermectin, mebendazole, mefloquine, meglumine antimoniate, melarsoprol, metrifonate, metronidazole,

niclosamide, nifurtimox, oxamniquine, paromomycin, pentamidine isethionate, piperazine, praziquantel, primaquine phosphate, proguanil, pyrantel pamoate, pyrimethanmine-sulfonamides, pyrimethanmine-sulfadoxine, quinacrine HCl, quinine sulfate, quinidine gluconate, spiramycin, stibogluconate sodium (sodium antimony gluconate), suramin, 5 tetracycline, doxycycline, thiabendazole, tinidazole, trimethoprim-sulfamethoxazole, and tryparsamide some of which are used alone or in combination with others.

Parasiticides used in non-human subjects include piperazine, diethylcarbamazine, thiabendazole, fenbendazole, albendazole, oxfendazole, oxibendazole, febantel, levamisole, pyrantel tartrate, pyrantel pamoate, dichlorvos, ivermectin, doramectin, milbemycin oxime, 10 iprinomectin, moxidectin, N-butyl chloride, toluene, hygromycin B thiacetarsemide sodium, melarsomine, praziquantel, epsiprantel, benzimidazoles such as fenbendazole, albendazole, oxfendazole, clorsulon, albendazole, amprolium; decoquinate, lasalocid, monensin sulfadimethoxine; sulfamethazine, sulfaquinoxaline, metronidazole.

Parasiticides used in horses include mebendazole, oxfendazole, febantel, pyrantel, 15 dichlorvos, trichlorfon, ivermectin, piperazine; for *S. westeri*: ivermectin, benzimidazoles such as thiabendazole, cambendazole, oxibendazole and fenbendazole. Useful parasiticides in dogs include milbemycin oxime, ivermectin, pyrantel pamoate and the combination of ivermectin and pyrantel. The treatment of parasites in swine can include the use of levamisole, piperazine, pyrantel, thiabendazole, dichlorvos and fenbendazole. In sheep and goats 20 anthelmintic agents include levamisole or ivermectin. Caparsolate has shown some efficacy in the treatment of *D. immitis* (heartworm) in cats.

Agents used in the prevention and treatment of protozoal diseases in poultry, particularly trichomoniasis, can be administered in the feed or in the drinking water and include protozoacides such as aminonitrothiazole, dimetridazole (Emtryl), nithiazide 25 (Hepzide) and Enheptin. However, some of these drugs are no longer available for use in agricultural stocks in the USA. Back yard flocks or pigeons not used for food production may be effectively treated with dimetridazole by prescription of a veterinarian (1000 mg/L in drinking water for 5-7 days).

The compounds of the invention can also be administered with other therapeutic 30 agents. For example, immune response agents such as growth factors and cytokines can be used in conjunction with the CpG oligonucleotides of the invention. In addition, activators of

innate immunity which synergize with CpG oligonucleotides, an example of which is GM-CSF, can also be co-administered in the methods of the invention. The compounds of the invention can be administered with IL-12 alone, IFN- γ alone, with both IL-12 and IFN- γ , or with any individual or combination of cytokines, chemokines or growth factors deemed
5 suitable for parasite infection control. "Immunopotentiating cytokines" are those molecules and compounds which stimulate the humoral and/or cellular immune response. The term "cytokine" is used as a generic name for a diverse group of soluble proteins and peptides which act as humoral regulators at nano- to picomolar concentrations and which, either under normal or pathological conditions, modulate the functional activities of individual cells and
10 tissues. These proteins also mediate interactions between cells directly and regulate processes taking place in the extracellular environment. Examples of cytokines useful according to the invention include, but are not limited to IL-1, IL-2, IL-6, IL-7, IL-12, IL-15, IL-18, granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), interferon- γ , (IFN- γ), tumor necrosis factor (TNF- α), Flt3 ligand, and CD40
15 ligand.

Flt3 ligand is a class of compounds described in EP0627487A2 and WO94/28391. A human Flt3 ligand cDNA was deposited with the American Tissue Type Culture Collection, Rockville, Maryland, and assigned accession number ATCC 69382. Interleukins (ILs) have been described extensively in the art, e.g., Mosley, et al., 1989, *Cell*, 59:335, Idzerda, et al.,
20 1990, *J. Exp. Med.*, 171:861. GM-CSF is commercially available as sargramostine, leukine (Immunex).

Cytokines play a role in directing the T cell response. Helper (CD4+) T cells orchestrate the immune response of mammals through production of soluble factors that act on other immune system cells, including other T cells. Most mature CD4+ T helper cells can
25 be classified as either Th1 or Th2 cells based on their cytokine release profiles. Th1 cells produce IL-2 and IFN- γ primarily as well as lower levels of IL-3, GM-CSF and TNF- α . The Th1 subset promotes delayed-type hypersensitivity, cell-mediated immunity, and immunoglobulin class switching to IgG_{2a}, in mouse, and to comparable IgG subclasses, in humans. The Th2 subset produces IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 and induces
30 humoral immunity by activating B cells, promoting antibody production, and inducing class switching to IgG₁ and IgE, in mouse for example.

In one embodiment, when CpG oligonucleotides are used to prevent or treat an existing parasite infection, a cytokine can also be administered along with the CpG oligonucleotide. In preferred embodiments, IL-12 or IFN- γ is the cytokine of choice. The co-administered cytokine(s) can act co-operatively, additively or synergistically with the CpG oligonucleotide of the invention to prevent parasite infection. The cytokine is administered in effective amounts. Such amounts of IL-12 or IFN- γ maybe less than those sufficient to provide a therapeutic benefit when the cytokine is administered alone and not in combination with a CpG oligonucleotide. A person of ordinary skill in the art would be able to determine the effective amounts needed. The term "co-administered," means administered proximally in time with another agent. By proximally in time, it is meant that a CpG oligonucleotide of the invention is administered to the subject close enough in time with the administration of the therapeutic agent (e.g., parasiticide, growth factor, cytokine, chemokine, etc.) to produce the desired biological effect.

Also envisioned in the present invention is the ability to target the CpG oligonucleotides to the specific immune cells such as macrophages, natural killer cells, granulocytes, dendritic cells or, in some cases, T cells in order to optimize the therapeutic and prophylactic effect. Alternatively, targeting infected tissues may be desirable, particularly if the delivery of the CpG oligonucleotide to the affected area would otherwise be slow or inefficient. Targeting motifs such as those described below may be directly conjugated to CpG oligonucleotides of the invention or they may be conjugated or linked to a carrier vehicle for the CpG oligonucleotide such as a lipid coating. Examples of agents that target specific cell types include ligands for cell surface receptors for individual cell types. An exemplary and non-limiting list is provided herein.

Exemplary receptors and preferred ligands useful in the targeting of the compounds of the invention include: hepatic receptors such as hyaluronic acid, collagen, N-terminal propeptide of collagen type III, mannose/N-acetylglucosamine, complement, asialoglycoprotein, tissue plasminogen activator, low density lipoprotein, insulin, ceruloplasmin, enterokinase, carcinoembryonic antigen, apamin, galactose/lactose; growth factor/cytokine receptors such as hepatocyte growth factor, epidermal growth factor, insulin-like growth factor I, II, interleukin-1a/b, interleukin-2, IL-7, IL-4, interferon- γ , interferon- β , keratinocyte growth factor, TNF-R p55; hormone receptors such as prolactin, thyroglobulin,

growth hormone, insulin, glucagon, leutinizing hormone, human choriogonadotrophic hormone; nerve cell receptors such as neurotensin; antigen presenting cell receptors such as immunoglobulin G-Fc receptor; kidney cell receptor/ligand targets such as angiotensin II, vasopressin; erythrocytes antigens such as c-kit, erythropoietin receptor; keratinocyte and
5 skin fibroblast receptors such as very low density lipoprotein, low density lipoprotein, integrins that bind to RGD bearing polypeptides, collagen, laminin; placental receptors such as hemopexin, immunoglobulin G-Fc, low density lipoprotein, transferrin, alpha2-macroglobulin, ferritin, insulin, interferon- γ , epidermal growth factor, insulin-like growth factor; muscle cell receptors such as insulin, very low density lipoprotein; and gut epithelium
10 receptors such as cobalamin-intrinsic factor, heat stable enterotoxin of E. Coli.

As discussed earlier, the compounds of the invention may also be associated with a molecule that results in higher affinity binding to the surface of an immune cell such as a dendritic cell. Similarly, the compounds can be associated with entities which increase their cellular uptake by target cells. Nucleic acids can be ionically, or covalently associated with
15 appropriate molecules using techniques which are well known in the art. A variety of coupling or crosslinking agents can be used, for example protein A, carbodiimide, and N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP). Nucleic acids can alternatively be encapsulated in liposomes or virosomes using well-known techniques.

According to the methods of the invention, the CpG oligonucleotide may also be
20 administered along with an antigen of parasitic origin or with an antibody or antibody fragment specific for either the parasite or the tissue affected by the infection. Although the CpG oligonucleotides are believed to function through an antigen non-specific immune response (i.e., an innate immune system response), antigens specific for parasites may also be used to induce a separate antigen specific immune response. The CpG oligonucleotides may
25 have an additional secondary effect which enhances an antigen-specific immune response. In addition, the CpG oligonucleotide could also be co-administered with an anti-idiotypic antibody. In general, pharmaceutically acceptable carriers for monoclonal antibodies, antibody fragments, and peptides are well-known to those of ordinary skill in the art. As used
30 herein, the terms "a pharmaceutically acceptable carrier" and "a therapeutically acceptable carrier" are used interchangeably and refer to a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. Pharmaceutically

acceptable carriers include diluents, fillers, salts, buffers, stabilizers, solubilizers and other materials which are well-known in the art. Exemplary pharmaceutically acceptable carriers for peptides in particular are described in U.S. Patent No. 5,211,657. The peptides of the invention may be formulated into preparations in solid, semi-solid, liquid or gaseous forms
5 such as tablets, capsules, powders, granules, ointments, solutions, depositories, inhalants and injections, and usual ways for oral, parenteral or surgical administration. The invention also embraces locally administering the compositions of the invention, including as implants.

A CpG oligonucleotide may be administered alone or in combination with the above-described therapeutic agent as part of a pharmaceutical composition. Such a pharmaceutical
10 composition may include the CpG oligonucleotide in combination with any standard physiologically and/or pharmaceutically acceptable carriers which are known in the art. The compositions should be sterile and contain a therapeutically effective amount of the CpG oligonucleotide in a unit of weight or volume suitable for administration to a subject. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible
15 solid or liquid filler, diluents or encapsulating substances which are suitable for administration into a human or other animal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that there is
20 no interaction which would substantially impair the desired pharmaceutical efficacy. Pharmaceutically acceptable further means a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. The characteristics of the carrier will depend on the route of administration. Physiologically and pharmaceutically acceptable carriers include diluents, fillers, salts, buffers, stabilizers, solubilizers, and other
25 materials which are well known in the art.

Compositions for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions. Preferably the compositions comprise a sterile aqueous preparation of the CpG oligonucleotide, which is isotonic with the bodily fluid, e.g., blood, of the recipient. This aqueous preparation may be formulated according to known methods
30 using suitable dispersing or wetting agents and suspending agents. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered

media. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables.

Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Those of skill in the art can readily determine the various parameters for preparing these alternative pharmaceutical compositions without resort to undue experimentation. Carrier formulations suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

The methods of the invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of the active compounds without causing clinically unacceptable adverse effects. Such modes of administration include oral, rectal, topical, mucosal, transdermal, subcutaneous, inhalation, intranasal, interdermal, or parenteral routes. The term "parenteral" includes subcutaneous, intravenous, intramuscular, or infusion. While intravenous or intramuscular routes may not particularly be suitable for long-term therapy and prophylaxis, they may be appropriate in emergency situations. Modes of administration which allow for self-administration such as oral and transdermal (i.e., in the form of a patch or an ointment or cream) are useful in the invention particularly for persons on vacation or military personnel who are unable to receive prompt medical attention. Oral or transdermal administration will be preferred for prophylactic treatment because of the convenience to the patient as well as in the ease of determining a dosing schedule. The compounds of the invention can take a liquid or solid form such as tablets and pills, or be present in an inhaled composition such as an aerosol. The compounds of the invention can also be administered using autoinjector devices such as those

currently used by military personnel. The CpG oligonucleotide may also be administered by gradual infusion over time. The particular mode selected will depend, of course, upon the particular infection or disease being treated and/or prevented, the particular drug selected, the severity of the condition being treated, and the dosage required for therapeutic efficacy. From
5 a practical point of view, a brief period of protection from malaria may be adequate for persons passing through or spending short periods of time in malaria-endemic areas. Even in cases where protection for longer than a week is desired, it is possible to give repeat administrations of CpG oligonucleotides. Delayed release, or long-term release in the form of sustained release devices including implants and transdermal patches are also envisioned as
10 suitable modes of administration. These latter devices are suitable for, among others, persons in chronic risk situations such as regions in which parasites are common. In an agricultural setting, either time release or simple modes of administration would be beneficial to livestock growers in order to rapidly prevent an infection in the entire flock or herd when at least one animal is suspected of being infected. In this event, CpG oligonucleotides should prove
15 simpler, less expensive and safer to use than repeated administrations of cytokines.

The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the CpG oligonucleotide into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by
20 uniformly and intimately bringing the CpG oligonucleotide into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the CpG oligonucleotide. Other compositions include suspensions in aqueous liquids or non-aqueous
25 liquids such as a syrup, elixir or an emulsion.

Unlike bacterial or viral infections, parasite infections do not induce immunological memory. As a result, a subject at risk of developing a parasite infection for an extended period of time can be administered CpG oligonucleotides using a long-term sustained, timed, or delayed release to prevent subsequent parasitic infections resulting from multiple
30 exposures. Long-term release, as used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient for at least 7 days, 10 days, 30

days, and preferably 60 days, and even more preferably for months or years. Such systems can avoid repeated administrations of the CpG oligonucleotide described above, increasing convenience to the subject and the physician. Long-term sustained release implants may also be particularly suitable for prevention in cases of chronic risk of infection or exposure. This mode of delivery is most preferred in the administration to individuals who are traveling abroad for extended periods of time without assurance of medical assistance. Additionally, persons living in developing or underdeveloped countries, or countries known to have a high incidence of particular parasitic infections are ideal subjects to receive long-term sustained release particularly given the sometimes nomadic lifestyles common in these countries.

Many types of sustained, delayed or timed release implants, including those that function in the long-term, are well-known to those of ordinary skill in the art. Some of the release systems include polymeric systems, as well as polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono- di- and tri-glycerides; hydrogel release systems; sytastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the CpG oligonucleotide is contained within a matrix such as those described in U.S. Patent Nos. 4,452,775, 4,675,189, and 5,736,152, and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,854,480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

One example of a delivery method for the compounds of the invention is a colloidal dispersion system. Colloidal dispersion systems include lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system of the invention is a liposome. Liposomes are artificial membrane vessels which are useful as a delivery vector *in vivo* or *in vitro*. It has been shown that large unilamellar vessels (LUV), which range in size from 0.2 - 4.0 μ can encapsulate large macromolecules. RNA, DNA, and

intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci.*, 1981, 6:77).

If desired, liposomes may be targeted to a particular tissue, such as the liver or any other such tissue affected by a parasite infection related disease. This is achieved by coupling
5 the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein capable of specifically recognizing a tissue of interest. Lipid formulations for transfection are commercially available from QIAGEN, for example as EFFECTENE™ (a non-liposomal lipid with a special DNA condensing enhancer) and SUPER-FECT™ (a novel acting dendrimeric technology) from Gibco BRL, for example, as LIPOFECTIN™ and
10 LIPOFECTACE™, which are formed of cationic lipids such as N-[1-(2, 3 dioleyloxy)-propyl]-N, N, N-trimethylammonium chloride (DOTMA) and dimethyl dioctadecylammonium bromide (DDAB). Methods for making liposomes are well known in the art and have been described in many publications. Liposomes also have been reviewed by Gregoriadis, G. in *Trends in Biotechnology*, V. 3, p. 235-241 (1985).

15 The invention embraces all modes of nucleic acid transfer which enhance or increase the efficiency of nucleic acid uptake into target cells of interest in vivo. Thus, the association of the CpG oligonucleotides of the invention to cationic lipids or dendrimers is included in the methods of the invention.

In one particular embodiment, the preferred vehicle is a biocompatible microparticle or
20 implant that is suitable for implantation into a vertebrate recipient. Exemplary bioerodible implants that are useful in accordance with this method are described in PCT International application no. PCT/US/03307 (Publication No. WO 95/24929, entitled "Polymeric Gene Delivery System", claiming priority to U.S. patent application serial no. 213,668, filed March 15, 1994). PCT/US/03307 describes a biocompatible, preferably biodegradable, polymeric
25 matrix for containing an exogenous gene under the control of an appropriate promoter. The polymeric matrix is used to achieve sustained release of the exogenous gene in a subject. In accordance with the instant invention, the CpG containing oligonucleotides described herein are encapsulated or dispersed within the biocompatible, preferably biodegradable polymeric matrix disclosed in PCT/US/03307. The polymeric matrix preferably is in the form of a
30 microparticle such as a microsphere (wherein the CpG oligonucleotide is dispersed throughout a solid polymeric matrix) or a microcapsule (wherein the CpG oligonucleotide is stored in the

core of a polymeric shell). Other forms of the polymeric matrix for containing the CpG oligonucleotide include films, coatings, gels, implants, and stents. The size and composition of the polymeric matrix device can be selected to result in favorable release kinetics in the tissue into which the matrix device is implanted. Alternatively, the implant may be designed
5 such that it releases sufficient levels of the CpG oligonucleotide to provide systemic exposure. The size of the polymeric matrix device can be further selected according to the method of delivery which is to be used, typically injection into a tissue or administration of a suspension by aerosol into the nasal and/or pulmonary areas. The polymeric matrix composition can be selected to have both favorable degradation rates and also to be formed of a material which is
10 bioadhesive, to further increase the effectiveness of transfer when the device is administered to a particular surface or tissue. The matrix composition also can be selected not to degrade, but rather, to release by diffusion over an extended period of time.

Both non-biodegradable and biodegradable polymeric matrices can be used to deliver the CpG oligonucleotide of the invention to the subject. Such polymers may be natural or
15 synthetic polymers. Synthetic polymers are preferred. The polymer is selected based on the period of time over which release is desired, generally in the order of a few hours to a year or longer. The period of sustained release will depend upon the subject and the environment. Typically, release over a period ranging from between a few hours and three to twelve months is most desirable. The polymer optionally is in the form of a hydrogel that can absorb up to
20 about 90% of its weight in water and further, optionally is cross-linked with multi-valent ions or other polymers.

In general, the CpG oligonucleotides of the invention are delivered using the bioerodible implant by way of diffusion, or more preferably, by degradation of the polymeric matrix. Exemplary synthetic polymers which can be used to form the biodegradable delivery
25 system include: polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terephthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, poly-vinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic esters, methyl
30 cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate

butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate),
5 poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), polyvinyl acetate, poly vinyl chloride, polystyrene and polyvinylpyrrolidone.

Examples of non-biodegradable polymers include ethylene vinyl acetate, poly(meth) acrylic acid, polyamides, copolymers and mixtures thereof.

Examples of biodegradable polymers include synthetic polymers such as polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, polyurethanes, poly(butic acid), poly(valeric acid), and poly(lactide-cocaprolactone), and natural polymers such as alginate and other polysaccharides including dextran and cellulose, collagen, chemical
15 derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water *in vivo*, by surface or bulk erosion.

Bioadhesive polymers useful in the invention include bioerodible hydrogels (described by H.S. Sawhney, C.P. Pathak and J.A. Hubell in *Macromolecules*, 1993, 26, 581-587, the teachings of which are incorporated herein), polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate),
25 poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate). Thus, the invention provides a composition of the above-described CpG oligonucleotide for use as a medicament, methods for preparing the medicament and methods for the sustained release of the medicament *in vivo*.

30 The materials for use in the invention, either in the administration of the compounds of the invention or in the measure of the bodily levels of these compounds or the factors they

induce, are ideally suited for the preparation of a kit. Such a kit may comprise a carrier means being compartmentalized to receive in close confinement one or more container means such as vials, tubes, and the like, each of the container means comprising one of the separate elements to be used in the method. For example, one of the container means may comprise a CpG
5 oligonucleotide of the invention. The kit may also have containers comprising other non-CpG oligonucleotide therapeutic agents useful in the invention as listed above. Additionally the kit may include containers for buffer(s) useful in the assay. If the mode of administration is by injection, the kit may also contain an injection delivery device such as an assembled needle and syringe or an autoinjector delivery device, such as those currently in use by the military.
10 Alternatively, the kit may be designed for subcutaneous injection and placement of a long-term sustained release capsule or implant, and would therefore contain an appropriate injection device such as for example a wide-bore needle for transfer of the capsule or implant to a subcutaneous region.

Other kits useful in the invention can comprise means for measuring the extent of the
15 immune response occurring in an individual, thereby indicating whether the individual is sufficiently primed to prevent a parasitic infection. For example, the kit can include means to measure cytokine levels. These kits can be used by the individual or more preferably by a physician, nurse or veterinarian. The kits can be useful in determining whether a long-term release device is continuing to emit the compounds of the invention or in assessing whether a
20 dose modification is necessary. If the kit is meant to measure cytokine or peptide levels in an individual, it will contain a readout system for measuring such a peptide. This readout system may comprise an antibody or other binding peptide which may be prepared on a solid surface such as polystyrene or may be applied to the surface at the time of individual testing. A bodily sample from an individual, preferably a liquid sample such as blood, can then be added
25 either directly or in diluted form onto the surface coated with binding peptide. The binding of components within the sample to the binding peptides of the kit can be measured by the use of a secondary binding peptide conjugated to a label. To be useful, the label should be directly or indirectly detectable or visible. A label which can be visualized using a colorimetric assay is most useful in the invention particularly if no additional instrumentation is required for
30 detection.

Examples

Materials and Methods

Oligonucleotides. The oligonucleotides used in this study were 1826, a 20-mer which contains two copies of a CpG motif known to have potent immunostimulatory effects on the murine immune system (TCCATGACGCTTCTCAGCGTT, CpG dinucleotides underlined for clarity, SEQ ID NO: 86), and 1982, a non-CpG control oligonucleotide (TCCAGGACTTCTCTCAGGTT, SEQ ID NO:92). The oligonucleotides were synthesized with a nuclease-resistant phosphorothioate backbone by Oligos Etc. (Wilsonville, OR). The Na⁺ salts of the oligonucleotide were ethanol precipitated and then resuspended in phosphate buffered saline (pH 7.2) and stored at 4 °C prior to injection. The endotoxin level in the oligonucleotides was undetectable (less than 1 ng/mg oligonucleotide) by Limulus assay (Whittaker Bioproducts, Walkersville, MD).

Parasites. *P. yoelii* (17X NL non-lethal strain, clone 1.1) was maintained by alternating passages of the parasites in *Anopheles stephensi* mosquitoes and CD-1 mice. Sporozoites were obtained from mosquito salivary glands 14 days after the mosquitoes had taken an infectious blood meal. Sporozoites were isolated from these mosquitoes by hand-dissection of the mosquito salivary glands in M199 medium containing 5% normal mouse serum (Rockland, Inc. Gilbertsville, PA) as previously described (Siu *et al.*, 1994). The recovered sporozoites were diluted to a final concentration of 250 infectious sporozoites per ml.

CpG Treatment and Challenge of Mice. All studies were carried out in 4 to 8 week old female BALB/c mice (Jackson Laboratory, Bar Harbor, ME), with 10 mice in each experimental group. Mice received a single injection into the tibialis anterior muscle of 3, 10, 50 or 100 µg of CpG oligonucleotide (1826) or of non-CpG control oligonucleotide (1982) in 50 µl saline at seven, two or one day(s) prior to sporozoite infection, or on the day of infection. Two separate studies were carried out several months apart, however all mice within the same study were challenged with sporozoites from the same stock at the same time. The first study comprised 9 groups (Table 2) and the second study comprised 13 groups (Table 3). To infect with sporozoites, each mouse was injected, at day 0, in the tail vein with 50 *P. yoelii* sporozoites in a volume of 200 µl. Blood was taken every other day between 4 and 14 days after infection by tail vein nicking (~10 µl) and Geimsa-stained blood films were prepared and examined microscopically for the presence of parasites. Protection was defined as the

complete absence of blood-stage parasitemia at any time between infection and day 14.

Treatment with anti-IFN- γ and anti-IL-12 monoclonal antibodies (mAbs). Some groups of mice receiving CpG oligonucleotide 2 days before challenge were also treated with mAbs to deplete IFN- γ or IL-12 in vivo. To deplete IFN- γ , mice received anti-IFN- γ mAb XMG-6 on
5 each of several days (Cherwinski *et al.*, 1987). In the first study (group 5), mice received an intraperitoneal (IP) injection of 1 mg of the anti-IFN- γ mAb on days -2, -1 and 0 relative to challenge. In the second study (group 12), mice received intravenous (IV) injection of 1 mg of the anti-IFN- γ mAb on each of days -3 and -2 and 1.5 mg on each of days -1, 0, +1, +2 and +4 relative to challenge (i.e., infection). To deplete IL-12, mice (first study, group 6) were
10 injected IP with 1 mg of the anti-IL12 mAb C17.8 (Cho *et al.*, 1996) at 12 hours prior to and 3 hours after infection.

Undepleted control groups were injected with purified rat Ig. In the first study (group 7), 1 mg rat Ig was given IP on each of days -2 and +2, and 2 mg on each of days -1 and 0 (relative to challenge). In the second study (group 13), the mice were administered by IV injection 1 mg
15 of rat Ig on each of days -3 and -2, and 1.5 mg on days -1, 0, +1, +2 and +4 relative to challenge.

Results

Effect of CpG Oligonucleotide on course of P. yoelii infection. Injection of untreated mice
20 with *P. yoelii* sporozoites resulted in infection (i.e., parasitemia) in 100% of mice within 14 days (Table 2, group 9; Table 3, group 22). Pre-treatment of mice with CpG oligonucleotide provided complete protection from infection when the CpG oligonucleotide was administered one or two days prior to challenge, as indicated by a complete absence of parasitemia up to 14 days after challenge (Table 2, groups 2 and 3; Table 3, group 10).

25 Protection was only partial when the time between CpG administration and sporozoites challenge was increased to 7 days (Table 2, group 1; Table 3, groups 14-17). In this case, doses of 3 to 100 μ g oligonucleotide provided 20-60% protection, with the higher levels of protection being associated with the higher doses of CpG (Table 3, groups 14-17). Mice receiving CpG oligonucleotides at the same time as sporozoite infection were not
30 protected at all (Table 2, group 4). There was never more than 20% protection (Table 3, groups 11 and 21), and in most groups no protection at all, in mice receiving control (non-

CpG) oligonucleotide (Table 2, group 8; Table 3, groups 11, 18-21). Moreover, this observed partial protection was not related to dose of non-CpG oligonucleotide since the two groups in which some mice did not become infected received 3 and 50 µg control oligonucleotide (Table 3, groups 11 and 21).

- 5 In total, these findings indicate that the protective effects seen with the CpG oligonucleotide were due to the immunostimulatory effects of the CpG motifs.

Role of IL-12 in protective effect of CpG Oligonucleotides. Mice administered with CpG Oligonucleotide 2 days before challenge and additionally treated with anti-IFN-γ mAb were only 80% protected compared to 100% of those similarly treated but not receiving anti-IFN-γ
10 (Table 2, Group 5). In contrast, there was a complete loss of protection in mice administered with CpG oligonucleotide 2 days before challenge and additionally treated with anti-IL-12 mAb (Table 2, Group 6). IP administration of rat Ig as a control had no effect on the CpG oligonucleotide-induced protection (Table 2, group 7).

Role of IFN-γ in protective effect of CpG Oligonucleotide. In the first study, there was little
15 evidence that IFN-γ played a role in the protective effects of the CpG oligonucleotide since 80% of mice treated with anti-IFN-γ and CpG oligonucleotide were still protected (Table 2, group 5). However, in that study, relatively low doses of anti-IFN-γ antibodies had been given by IP injection over a relatively short period of time (days -2 to 0). In the second study, higher doses of anti-IFN-γ antibodies were given IV over a longer period (days -3 to +4), and
20 in this case the loss of protection was more evident with only 20% of mice failing to become infected (Table 3, group 12), the same as with the control oligonucleotide (Table 3, group 11). IV administration of rat Ig as a control had no effect on the CpG oligonucleotide-induced protection (Table 3, group 13).

Table 2. Effect of CpG Oligonucleotide on malaria infection in mice - Study I

| 5 | <u>Group</u> | <u>Treatment</u> | <u>Day CpG ODN</u> | <u>No. infected/</u> | <u>% Protection**</u> |
|----|--------------|---------------------------------|----------------------|----------------------|-----------------------|
| | | | <u>Administered*</u> | <u>No. total</u> | |
| | 1 | CpG ODN | -7 | 4/10 | 60 |
| | 2 | CpG ODN | -2 | 0/10 | 100 |
| | 3 | CpG ODN | -1 | 0/10 | 100 |
| 10 | 4 | CpG ODN | 0 | 10/10 | 0 |
| | 5 | CpG ODN + anti-IFN- γ | -2 | 2/10 | 80 |
| | 6 | CpG ODN + anti-IL-12 | -2 | 10/10 | 0 |
| 15 | 7 | CpG ODN + Ctrl Ig | -2 | 0/10 | 100 |
| | 8 | Control ODN | -2 | 10/10 | 0 |
| | 9 | None | N/A | 18/18 | 0 |

20 * Day administered relative to intravenous inoculation of sporozoites (i.e., day 0).

** Based on more than a decade's experience working with the *P. yoelii* sporozoite infection model in mice, any mouse with a negative blood smear on day 14 is considered to be protected.

N/A = not applicable

25 ODN = oligonucleotide

Table 3. Effect of CpG ODN on malaria infection in mice - Study 2

| | <u>Group</u> | <u>Treatment</u> | <u>Dose ODN (ug)</u> | <u>Day ODN Administered*</u> | <u>No. infected/ No. total</u> | <u>% Protection**</u> |
|----|--------------|----------------------|------------------------------|----------------------------------|------------------------------------|---------------------------|
| 5 | 10 | CpG ODN | 50 | -2 | 0/10 | 100 |
| | 11 | Control ODN | 50 | -2 | 8/10 | 20 |
| | 12 | CpG ODN + | | | | |
| 10 | | anti-IFN- γ | 50 | -2 | 8/10 | 20 |
| | 13 | CpG ODN + Ctrl Ig | 50 | -2 | 0/10 | 100 |
| | 14 | CpG ODN | 100 | -7 | 2/10 | 80 |
| | 15 | CpG ODN | 50 | -7 | 5/10 | 50 |
| 15 | 16 | CpG ODN | 10 | -7 | 7/10 | 30 |
| | 17 | CpG ODN | 3 | -7 | 7/10 | 30 |
| | 18 | Control ODN | 100 | -7 | 10/10 | 0 |
| | 19 | Control ODN | 50 | -7 | 10/10 | 0 |
| | 20 | Control ODN | 10 | -7 | 10/10 | 0 |
| 20 | 21 | Control ODN | 3 | -7 | 8/10 | 20 |
| | 22 | none | N/A | N/A | 6/6 | 0 |

* Day administered relative to intravenous inoculation of sporozoites (i.e., day 0).

** Based on more than a decade's experience working with the *P. yoelii* sporozoite

infection model in mice, any mouse with a negative blood smear on day 14 is considered to be protected.

N/A = not applicable

ODN = oligonucleotide

It should be understood that the preceding is merely a detailed description of certain preferred embodiments. It therefore should be apparent to those skilled in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than
5 routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

All references, patents and patent publications that are recited in this application are incorporated in their entirety herein by reference.

What is claimed is presented below and is followed by a Sequence Listing.

10 We claim:

Claims

1. A method for preventing a parasitic infection in a subject comprising:
administering to a subject at risk of being infected with a parasite an effective amount,
for preventing a parasitic infection, of an oligonucleotide having a sequence including at least
5 the following formula:



wherein the oligonucleotide includes at least 6 nucleotides wherein C and G are
unmethylated and wherein X_1 and X_2 are nucleotides,
prior to exposure to a parasite.

10

2. The method of claim 1, wherein the oligonucleotide is 6 to 100 nucleotides in length.
3. The method of claim 1, wherein X_1 is selected from the group of A, T or G.
- 15 4. The method of claim 1, wherein X_2 is selected from the group consisting of A, C or T.
5. The method of claim 1, wherein the subject is human or non-human.
6. The method of claim 1, wherein the subject is selected from the group consisting of a
20 cat, dog, cow, pig, sheep, horse, chicken, duck, goose, fish, goat, mouse, rat, gerbil, rabbit and
a zoo animal.
7. The method of claim 1, wherein the parasite is an intracellular parasite.
- 25 8. The method of claim 1, wherein the parasite is an obligate intracellular parasite.
9. The method of claim 1, wherein the parasite is selected from the group consisting of
Plasmodium falciparum, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium vivax*,
Plasmodium knowlesi, *Babesia microti*, *Babesia divergens*, *Trypanosoma cruzi*, *Toxoplasma*
30 *gondii*, *Trichinella spiralis*, *Leishmania major*, *Leishmania donovani*, *Leishmania braziliensis*
and *Leishmania tropica*.

10. The method of claim 1, wherein the parasite is selected from the group consisting of *Trypanosoma gambiense*, *Trypanosoma rhodesiense* and *Schistosoma mansoni*.
- 5 11. The method of claim 1, wherein the parasite causes malaria.
12. The method of claim 1, wherein the subject is also administered an effective amount of one or more non-CpG oligonucleotide therapeutic agents.
- 10 13. The method of claim 12, wherein the non-CpG oligonucleotide therapeutic agent is a parasiticide.
14. The method of claim 12, wherein the non-CpG oligonucleotide therapeutic agent is selected from the group consisting of IL-1, IL-6, IL-12, IL-15, IL-18, IFN- γ , TNF- α , GM-
15 CSF, CD40 ligand and Flt3 ligand.
- 15.. The method of claim 14, wherein IL-12 and IFN- γ are administered and IL-12 is administered prior to IFN administration.
- 20 16. The method of claim 1, wherein the oligonucleotide is administered more than once.
17. The method of claim 1, wherein the oligonucleotide is administered at least 7 days prior to a parasite infection.
- 25 18. The method of claim 1, wherein the oligonucleotide is administered at least 2 days prior to a parasite infection.
19. The method of claim 1, wherein the oligonucleotide is administered at least 24 hours prior to a parasite infection.
- 30 20. The method of claim 1, wherein the oligonucleotide is administered orally, mucosally,

transdermally, subcutaneously, parenterally, or by inhalation.

21. The method of claim 1, wherein the oligonucleotide is administered in a sustained release vehicle.

5

22. The method of claim 21, wherein the sustained release vehicle is a liposome.

23. A pharmaceutical preparation comprising the oligonucleotide of claim 1, at least one therapeutic agent and a therapeutically acceptable carrier.

10

24. A kit comprising the pharmaceutical preparation of claim 23 in at least one container and instructions for use wherein the oligonucleotide and the therapeutic agent are in separate containers.

15 25. A sustained release device comprising the oligonucleotide of claim 1 and a polymer capable of release for at least 7 days.

26. A sustained release device comprising the oligonucleotide of claim 1 and a polymer capable of release for at least 10 days.

20

27. A sustained release device comprising the oligonucleotide of claim 1 and a polymer capable of release for at least 30 days.

28. A sustained release device comprising the oligonucleotide of claim 1 and a polymer
25 capable of release for at least 60 days.

29. A method for treating a subject infected with a parasite comprising:
administering to the subject an effective amount for treating a parasite infection of an
oligonucleotide having a sequence including at least the following formula:

30



wherein the oligonucleotide includes at least 6 nucleotides wherein C and G are

unmethylated and wherein X_1 and X_2 are nucleotides.

30. The method of claim 29, wherein the oligonucleotide is 6 to 100 nucleotides in length.

5 31. The method of claim 29, wherein X_1 is selected from the group of A, T or G.

32. The method of claim 29, wherein X_2 is selected from the group consisting of A, C or T.

33. The method of claim 29, wherein the subject is human or non-human.

10

34. The method of claim 29, wherein the subject is selected from the group consisting of a cat, dog, cow, pig, sheep, horse, chicken, duck, goose, fish, goat, mouse, rat, gerbil, rabbit and a zoo animal.

15 35. The method of claim 29, wherein the parasite is an intracellular parasite.

36. The method of claim 29, wherein the parasite is an obligate intracellular parasite.

37. The method of claim 29, wherein the parasite is selected from the group consisting of
20 *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium vivax*,
Plasmodium knowlesi, *Babesia microti*, *Babesia divergens*, *Trypanosoma cruzi*, *Toxoplasma gondii* and *Trichinella spiralis*.

38. The method of claim 29, wherein the parasite is selected from the group consisting of
25 *Trypanosoma gambiense*, *Trypanosoma rhodesiense* and *Schistosoma mansoni*.

39. The method of claim 29, wherein the parasite causes malaria.

40. The method of claim 29, wherein the subject is also administered an effective amount of
30 one or more non-CpG oligonucleotide therapeutic agents.

41. The method of claim 40, wherein the non-CpG oligonucleotide therapeutic agent is a parasiticide agent.

42. The method of claim 40, wherein the non-CpG oligonucleotide therapeutic agent is
5 selected from the group consisting of IL-1, IL-6, IL-12, IL-15, IL-18, IFN- γ , TNF- α , GM-CSF, CD40 ligand and Flt3 ligand.

43. The method of claim 42, wherein IL-12 and IFN- γ are administered and IL-12 is administered prior to IFN administration.

10

44. The method of claim 29, wherein the oligonucleotide is administered more than once.

45. The method of claim 29, wherein the oligonucleotide is administered within 24 hours of parasite infection.

15

46. The method of claim 29, wherein the oligonucleotide is administered within 48 of parasite infection.

47. The method of claim 29, wherein the oligonucleotide is administered within 7 days of
20 parasite infection.

48. The method of claim 29, wherein the oligonucleotide is administered orally, mucosally, transdermally, subcutaneously, parenterally, or by inhalation.

49. The method of claim 29, wherein the oligonucleotide is administered in a sustained
25 release vehicle.

50. The method of claim 49, wherein the sustained release vehicle is a liposome.

30

Abstract

A method for preventing parasitic infections and infection related diseases by the administration of a CpG oligonucleotide prior to parasite exposure to a subject at risk of parasite exposure is provided. The invention also provides methods for treating subjects having parasitic
5 infections by the administration of CpG oligonucleotides after parasite exposure.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Robert A. Gramzinski, et al.,
Serial No. : Not Yet Assigned
Filing Date : Herewith
For : METHODS FOR THE PREVENTION AND TREATMENT OF
PARASITIC INFECTIONS AND RELATED DISEASES USING CpG
OLIGONUCLEOTIDES

Box PATENT APPLICATION
Assistant Commissioner of Patents
Washington, D.C. 20231

STATEMENT PURSUANT TO 37 CFR 1.821(f)

This statement is made pursuant to 37 CFR 1.821(f). Applicant encloses herewith an original copy of the Sequence Listing and a computer readable diskette. Applicant's agent states that the content of the paper copy of the Sequence Listing and the computer readable diskette contain the identical information and contain no new matter.

Respectfully submitted,



Helen C. Lockhart, Reg. No. 39,248
Wolf, Greenfield & Sacks, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210
Tel. (617) 720-3500

Attorney Docket No. C1039/7027
Date: May 6, 1999
x05/6/99

-1-

SEQUENCE LISTING

<110> Gramzinski, Robert A.
Krieg, Arthur M.
Davis, Heather L.
Hoffman, Stephen L.

<120> Methods for the Prevention and Treatment
of Parasitic Infections and Related Diseases Using CpG
Oligonucleotides

<130> C1039/7027/HCL

<150> US 60/084,512

<151> 1998-05-06

<160> 92

<170> FastSEQ for Windows Version 3.0

<210> 1

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 1

gctagacgtt agcgt

15

<210> 2

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 2

gctagatggt agcgt

15

<210> 3

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<221> modified_base

<222> (13)...(13)

<223> m5c

<400> 3

gctagacggtt agcgt

15

<210> 4
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 4
gcatgacggtt gagct

15

<210> 5
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 5
atggaaggctc cagcgttctc

20

<210> 6
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 6
atcgactctc gagcgttctc

20

<210> 7
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<221> modified_base
<222> (3)...(3)
<223> m5c

<400> 7
atcgactctc gagcgttctc

20

<210> 8
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<221> modified_base
<222> (18)...(18)
<223> m5c

<400> 8
atcgactctc gacggttctc 20

<210> 9
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 9
atcgactctc gaacgttctc 20

<210> 10
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 10
gagaacgctg gaccttccat 20

<210> 11
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 11
gagaacgctc gaccttccat 20

<210> 12
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 12
gagaacgctc gaccttcgat 20

<210> 13
<211> 20
<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 13
gagcaagctg gaccttccat 20

<210> 14
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<221> modified_base
<222> (14)...(14)
<223> m5c

<400> 14
gagaacgctg gaccttccat 20

<210> 15
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 15
gagaacgatg gaccttccat 20

<210> 16
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 16
gagaacgctc cagcactgat 20

<210> 17
<211> 19
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 17
ccatgtcggg cctgatgct 19

<210> 18
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<221> modified_base
<222> (12)...(12)
<223> m5c

<400> 18
tccatgtcgg tcctgatgct

20

<210> 19
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 19
tccatgacgt tcctgatgct

20

<210> 20
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 20
tccatgtcgg tcctgacgca

20

<210> 21
<211> 8
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 21
tcaacggt

8

<210> 22
<211> 8
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

| | |
|---------------------------|---|
| <400> 22 | |
| tcagcgct | 8 |
| <210> 23 | |
| <211> 8 | |
| <212> DNA | |
| <213> Artificial Sequence | |
| <220> | |
| <223> Synthetic Sequence | |
| <400> 23 | |
| tcttcgat | 8 |
| <210> 24 | |
| <211> 8 | |
| <212> DNA | |
| <213> Artificial Sequence | |
| <220> | |
| <223> Synthetic Sequence | |
| <400> 24 | |
| tcttcgaa | 8 |
| <210> 25 | |
| <211> 7 | |
| <212> DNA | |
| <213> Artificial Sequence | |
| <220> | |
| <223> Synthetic Sequence | |
| <400> 25 | |
| caacggt | 7 |
| <210> 26 | |
| <211> 8 | |
| <212> DNA | |
| <213> Artificial Sequence | |
| <220> | |
| <223> Synthetic Sequence | |
| <400> 26 | |
| ccaacggt | 8 |
| <210> 27 | |
| <211> 9 | |
| <212> DNA | |
| <213> Artificial Sequence | |
| <220> | |
| <223> Synthetic Sequence | |
| <400> 27 | |

caacgttct

9

<210> 28
 <211> 8
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic Sequence

<400> 28
 tcaacgtc

8

<210> 29
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic Sequence

<400> 29
 atggactctc cagcgttctc

20

<210> 30
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic Sequence

<400> 30
 ataggaggtc caacgttctc

20

<210> 31
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic Sequence

<400> 31
 atcgactctc gagcgttctc

20

<210> 32
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic Sequence

<400> 32
 atggaggctc catcgttctc

20

<210> 33
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<221> modified_base
<222> (14)...(14)
<223> m5c

<400> 33
atcgactctc gagcgttctc

20

<210> 34
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 34
gcatgacggt gagct

15

<210> 35
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 35
tccatgtcgg tcctgatgct

20

<210> 36
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 36
tccatggcgg tcctgatgct

20

<210> 37
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 37
tccatgacgg tcctgatgct 20

<210> 38
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 38
tccatgtcga tcctgatgct 20

<210> 39
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 39
tccatgtcgc tcctgatgct 20

<210> 40
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 40
tccatgtcgt tcctgatgct 20

<210> 41
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 41
tccataacgt tcctgatgct 20

<210> 42
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 42

tccatgacgt ccctgatgct

20

<210> 43
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 43
tccatcacgt gcctgatgct

20

<210> 44
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 44
ggggtcaacg ttgacgggg

19

<210> 45
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 45
ggggtcagtc gtgacgggg

19

<210> 46
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 46
gctagacgtt agtgt

15

<210> 47
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 47
tccatgtcgt tcctgatgct

20

<210> 48
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 48
accatggacg atctgtttcc cctc

24

<210> 49
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 49
tctcccagcg tgcgccat

18

<210> 50
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 50
taccgcgtgc gaccctct

18

<210> 51
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 51
accatggacg aactgtttcc cctc

24

<210> 52
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 52
accatggacg agctgtttcc cctc

24

<210> 53

<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 53
accatggacg acctgtttcc cctc 24

<210> 54
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 54
accatggacg tactgtttcc cctc 24

<210> 55
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 55
accatggacg gtctgtttcc cctc 24

<210> 56
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 56
accatggacg ttctgtttcc cctc 24

<210> 57
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 57
cacgttgagg ggcat 15

<210> 58
<211> 12

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 58
tcagcgtgcg cc 12

<210> 59
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 59
atgacgttcc tgacgtt 17

<210> 60
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 60
tctcccagcg ggcgcatt 17

<210> 61
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 61
tctcccagcg cgcgccat 18

<210> 62
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 62
tccatgtcgt tctgtcgtt 20

<210> 63
<211> 20
<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 63
tccatagcgt tcctagcgtt 20

<210> 64
<211> 21
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 64
tcgtcgctgt ctccgcttct t 21

<210> 65
<211> 19
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 65
tcctgacggt cctgacggt 19

<210> 66
<211> 19
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 66
tcctgtcggt cctgtcggt 19

<210> 67
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 67
tccatgtcgt tttgtcgtt 20

<210> 68
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 68
tcctgtcggt cctgtcggt 20

<210> 69
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 69
tcctgtcggt tcctgtcggt 20

<210> 70
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 70
tcctgtcggt tttgtcggt 20

<210> 71
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 71
tcgtcggtgt ctgcccttct t 21

<210> 72
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 72
tcgtcggtgt tgctgtttct t 21

<210> 73
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 73
tccatgcgtg cgtgcgtttt 20

<210> 74
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 74
tccatgcgtt gcgttgcgtt 20

<210> 75
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 75
tccacgacgt ttcgacgtt 20

<210> 76
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 76
tcgtcgttgt cgttgcgtt 20

<210> 77
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 77
tcgtcgtttt gtcgttttgt cgtt 24

<210> 78
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 78
tcgtcggtgt cgtttgtcg tt 22

<210> 79
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 79
gcgtgcgttg tcgttgtcgt t 21

<210> 80
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 80
tgtcgttgt cgtttgtcgt t 21

<210> 81
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 81
tgtcgttgtc gttgtcgttg tcgtt 25

<210> 82
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 82
tgtcgttgtc gttgtcgtt 19

<210> 83
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 83

| | |
|---------------------------|----|
| tcgtcgtcgt cggt | 14 |
| <210> 84 | |
| <211> 13 | |
| <212> DNA | |
| <213> Artificial Sequence | |
| <220> | |
| <223> Synthetic Sequence | |
| <400> 84 | |
| tgctggtgtc gtt | 13 |
| <210> 85 | |
| <211> 20 | |
| <212> DNA | |
| <213> Artificial Sequence | |
| <220> | |
| <223> Synthetic Sequence | |
| <400> 85 | |
| tccatagcgt tcctagcgtt | 20 |
| <210> 86 | |
| <211> 20 | |
| <212> DNA | |
| <213> Artificial Sequence | |
| <220> | |
| <223> Synthetic Sequence | |
| <400> 86 | |
| tccatgacgt tcctgacgtt | 20 |
| <210> 87 | |
| <211> 6 | |
| <212> DNA | |
| <213> Artificial Sequence | |
| <220> | |
| <223> Synthetic Sequence | |
| <400> 87 | |
| gtcgyt | 6 |
| <210> 88 | |
| <211> 7 | |
| <212> DNA | |
| <213> Artificial Sequence | |
| <220> | |
| <223> Synthetic Sequence | |
| <400> 88 | |
| tgctggt | 7 |

<210> 89
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 89
tctcccagcg tgcgcat

18

<210> 90
<211> 6
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 90
gtcgct

6

<210> 91
<211> 7
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 91
tgtcgct

7

<210> 92
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 92
tccaggactt ctctcagggtt

20